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The Effects Of Estrogen, Progesterone And Ionized Calcium On Seizures During The Menstrual Cycle Of Mature Female Epileptics

John Jacono

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THE EFFECTS OF ESTROGEN, PROGESTERONE AND IONIZED CALCIUM ON SEIZURES DURING THE MENSTRUAL CYCLE OF MATURE FEMALE EPILEPTICS

by

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Submitted in partial fulfillment
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ABSTRACT

More males than females are affected by epilepsy. This is so for all ages, with the exception of puberty. During the pubertal years, incidence rates of epilepsy are higher in females than in males.

Factors suspected of causing epilepsy are numerous, however the role of the female sex hormone estrogen is thought to be highly significant to epilepsy of puberty.

This study was designed to provide information about the role of estrogen and ionized calcium in seizures during the menstrual cycle of gynaecologically mature female epileptics. The study sample comprised 16 female epileptics ages 18 to 40 years resident in southwestern Ontario. Each subject donated a blood sample on alternate days for the duration of one menstrual cycle. In addition, seizures occurring were entered on a seizure diary.

Results were analyzed using linear regression techniques adjusted for repeated measurements and measures of association adjusted for clustering effect.

It was found that high levels of estrogen encountered during the middle of the cycle severely decreased levels of ionized calcium at that time.

Further, most of the seizures exhibited by the subjects occurred before and after the estrogen peak when ionized calcium levels were relatively stable. Thus ionized calcium levels appear to have a salutary effect on estrogen and may reduce seizures expected at this time.

No causal inferences are generated from these results. Rather,

an expansion of previously promoted models of epileptogenesis is proposed. Increased hypothalamic work rate to stimulate production/reduction of hormones governing the menstrual cycle, the presence of fluctuating levels of estrogen and concomitant alteration in the ionic micro-environment could lead to the spread of intense burst firing activity. This model is applicable to both pubertal as well as mature females.

ACKNOWLEDGEMENTS

This study owes its inception and completion to the generous support of the Physicians' Services Incorporated foundation to whom I am deeply grateful.

Hours of discussion, encouragement and trust contributed by Drs. Blume, Girvin and Rand helped transform a curiosity into its present form. Much practical help was also contributed by the clinical neurological sciences staff at the three London teaching hospitals.

No amount of help, however, would have brought this work to conclusion without the co-operation of the subjects and their guardians. A special vote of thanks then must go above all to the subjects themselves. Dr. Lockwood, Mr. Stogdill and the staff of the Oxford Regional Centre, with Dr. Pearce, Ms. Murphy and the staff of the South Western Regional Centre worked assiduously on my behalf. In addition, I cannot help but salute the parents of my subjects for their trust in me, and the hope that their daughters' sacrifice might prove beneficial to others in the future.

The time spent by my committee members in challenging, encouraging and advising me will not be forgotten. My principal advisor, Dr. J. McD. Robertson, was there when I needed him. He had above all the presence of mind to steer me clear of my obsessions when things got hectic, by entrapping me in my favourite topic of conversation - trout fishing. Drs. Wanklin and Blume spent much time critiquing my work and guiding me back to the task at hand.

Feelings of loneliness sometimes verging on despair are frequent visitors to the life of the graduate student. My experience was no different. The camaraderie and practical help offered by my fellow

students was of great benefit to me. Some have gone on to become my trusted friends. To Dr. Alan Donald, Dr. Gail Frankel, Ms. Sandra Isaacs, Mr. Scott MacDonald and Ms. Pat Petryshen, my thanks - again. Special thanks also go to Andrew John MacRobert and Carol Ann Ross (these two love double-barrelled monickers) who looked after my psyche and my body with great sensitivity and forebearance in the final weeks. The departmental staff of Ms. Helen Simpson and Mrs. Elma DaCosta rendered me yeoman service in addition to constant encouragement,

Finally, my deepest thanks to my daughter Anna Clare. Her pride stimulated the vainglorious in me which provided welcome rays of light in my dark days. To her an apology for not being there in her times of need. We have grown a little bit older and maybe even a little bit wiser. Now that this is all over we can contribute more to each other. To her this work is dedicated.

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AIMS OF STUDY

1.1 Introduction

The prevalence of epilepsy in the population approximates 7 per 1,000 persons - so the number afflicted with this disorder can not be considered inconsequential. The cost in diagnosis, chemotherapeutic and/or surgical interventions when added to the need for financial and social assistance, place a considerable burden on any country's health care budget. Potential loss in contributions to the community further adds to the national economic burden. The personal psycho-social losses endured by these people remain high, in spite of a more enlightened and presumably more tolerant society.

Incidence rates for epilepsy in the female population increase dramatically during puberty - around the latter part of the first, and the early part of the second decade of life. At all other ages, female incidence rates are consistently lower than those of their male counterparts. Factors contributing to this female excess in incidence rates during puberty remain largely unknown. However, peripheral research suggests that the action of sex steroids may play an important role. These steroids exert both a direct effect on the brain, as well as indirect effects through interactions with other minerals, enzymes and electrolytes.

The effects of estrogen on the central nervous system have been commented on since the late nineteenth century. Much of this earlier knowledge about its relationship to epilepsy was derived indirectly through observation of the effects of puberty and the menstrual cycle.

Later, its effects were confirmed directly and estrogen is currently used topically to stimulate burst firing activity in experimental research. Estrogen's effect on calcium owes its discovery to research on osteoporosis in the post-menopausal female. In 1978, Pitkin et al. demonstrated the cyclical nature of estrogen's antagonistic action on calcium resorption during the menstrual cycle.

Much work has been done on the effects of different levels of ionized calcium on the central nervous system. Its significant role in neurotransmission has been reported since the 1920's. Later research has only served to confirm the complexity of the central nervous system. Necessary for brain function, ionized calcium in decreased levels can severely disturb endogenous systems of control, resulting in burst firing activity. Its enigmatic nature remains unresolved today.

The effects of estrogen on the central nervous system, estrogen on calcium levels and the effects of ionized calcium levels on the central nervous system have promoted many hypotheses and models. However, no one unitary model exists. This only confirms the multicausal nature of central nervous system dysfunction. In addition, much of what is known has been derived from research carried out on lower species, sometimes at the unicellular level. Human observational research may help confirm past animal based models, or it may promote other models for further research.

Any attempt at enquiry into the role of these steroids and their interaction in epilepsy in the female during the peri-pubertal period would have to involve a large cohort of pre-pubescent females. This cohort would have to be followed over a lengthy prospective study during which various variables would be studied. Clearly the cost

factor makes it unfeasible at this time.

However, the same steroid/mineral/enzyme interactions that take place during the female peri-pubertal period appear to be repeated cyclically during the menstrual cycle. Thus the use of gynaecologically mature females could still render valuable inferential results. These results would supply the impetus necessary for further research, either to confirm the validity of previous animal based research and models, or to further test emergent hypotheses.

1.2 Aims of Study

(a) Above average seizuring around specific stages of the menstrual cycle (predominantly but not solely catamenial in nature) has been reported in the past by a variety of researchers. This study sought to investigate the possible relationship of menstrual cycle stage to seizuring activity in the study population.

(b) The epileptogenic nature of estrogen and conversely the anti-convulsant properties of progesterone have been demonstrated before. In addition, an inverse relationship between estrogen and ionized calcium has been postulated. Experimentally, a low ionized calcium milieu has been shown to provoke spontaneous burst firing activity in excitable cells.

This study attempts to establish a relationship between estrogen, progesterone, ionized calcium levels and seizure activity during the menstrual cycle of epileptic subjects in the study.

Chapter 2

LITERATURE REVIEW

2.1 Epidemiology of Epilepsy

Epilepsy, defined by Glaser (1980) as "... many types of recurrent seizures produced by paroxysmal excessive neuronal discharges in different susceptible parts of the brain, that can be due to a variety of cerebral and general bodily disorders.", is one of the most common disorders of the central nervous system (C.N.S.). Although it can take many forms, the term tends to conjure in most laymens' minds its most dramatic manifestation - that of a grand mal seizure. Not surprisingly then epilepsy has been a stigmatising disorder for millenia. Fear of epilepsy and thus of the epileptic remains high even today. Those afflicted with this disorder continue to go to great lengths to hide their affliction. Compounding this problem are ongoing disagreements about diagnosis and seizure classification. Together, these two factors have made ascertainment of rates of epilepsy very difficult indeed.

From thirty published epidemiologic studies of epilepsy, Rose et al. (1973) demonstrated the great variability in reported rates. Prevalence rates ranged from a low of 1.5 per 1,000 (Ueki and Sato, 1963) to a high of 7.4 per 1,000 (Levy, 1970). These reported differences reflect problems with case ascertainment, inadequate record keeping, haphazard data reporting and subject selection biases (not always declared). Thus reported rates can mask not only severe methodological deficiencies but also - and to unknown degree - real geographic, age, sex and ethnic differences.

The Hauser and Kurland (1975) study of incidence and prevalence of epilepsy in Rochester, Minnesota has however become the benchmark for

comparison. Although it lacks data on incidence and prevalence of epilepsy among blacks and those of Hispanic origin, this study derives its strength from its duration (33 years). The presence of the Mayo Clinic, the only facility with resident neurologists and diagnostic capacity in the area, contributed greatly to case ascertainment. Clear and consistent inclusion criteria and data categorization further added to the reliability of data published in this study. Hauser and Kurland (1975) reported a Mean Incidence rate of 48.7 per 100,000 per annum, and a Mean Prevalence rate of 6.5 per 1,000 of the population.

2.2 Reported Rates

2.2.1 Introduction

The estimation of prevalence rates for epilepsy may require the examination of patient records from a lengthy period. The disorder has a low incidence rate and remissions of several years are not uncommon. Shorvon (1984) has argued that the widely held belief that "once an epileptic, always an epileptic" was erroneous and based on epidemiologic studies of captive institutionalised populations. He further suggested that a more realistic remission rate for the whole population would approximate 70%. Sofijanov (1982) reviewed various published reports and subdivided her data into three categories. What she termed pessimistic rates - ranging between 20 and 30% remissions - were published by Bridge (1949), Alström (1950) and Rodin (1968). Moderate remission rates - between 40 and 60% - were reported by Livingston (1963), Breg and Yannet (1962), Jeras and Tividar (1973), Sillanpaa (1973), Hauser et al. (1973), Annegers et al. (1979) and Okuma and Kamashiro (1981). Excellent rates of remission (80%) were reported by Boshes and Kienast (1970). Hauser and Kurland (1975) used strict

remission criteria (five years of seizure and anticonvulsant-free living). They found a time dependent increase in remission rates among their Rochester subjects. A 10% remission rate observed in their 1955-1959 cohort increased to 29% in the 1935-1944 group. These two rates are significantly different from the proposed Shovron (1984) rates; they are in fact equivalent to the rates Sofijanov termed pessimistic. Hauser and Kurland (1975) found no significant sex-specific differences in rates of remission.

Epileptics in remission may unilaterally terminate medical supervision and treatment, while others with a low seizure incidence (and thus not in remission) may also decide that treatment had not appreciably altered their seizure frequency. The stigmatising nature of epilepsy may stop some from seeking treatment (especially if they do not suffer from grand-mal epilepsy). Mislabelling (not necessarily misdiagnosing), as a humanitarian gesture protective of the individual, is also not inconceivable. All these individuals would then be excluded from the calculation of rates. The magnitude of this problem, by definition, cannot be ascertained.

The other side of the problem in the calculation of prevalence rates involves the previously mentioned disagreement among diagnosticians and researchers, about subject inclusion or classification. Children experiencing febrile convulsions are often included in the calculation of rates. The percentage of children with febrile convulsions who go on to experience epilepsy on a chronic basis varies considerably. Kurland (1960) reported that 25% of children experiencing febrile convulsions develop epilepsy later. Freeman (1980) reported a 13% rate, Frierichen and Melchior (1954), Livingston (1958), Millichap

(1968), van den Berg and Yerushalmy (1969) all reported a rate of approximately 5%, while Hauser and Kurland (1975) reported the smallest rate of exacerbation of symptoms - 2.3%.

Inclusion of individuals (especially when undeclared or categorised exclusively), who exhibit only febrile convulsions or only one seizure as a result of systemic metabolic dysfunction, could dramatically inflate both incidence as well as prevalence rates. Reported rates from various studies must then be examined from these perspectives.

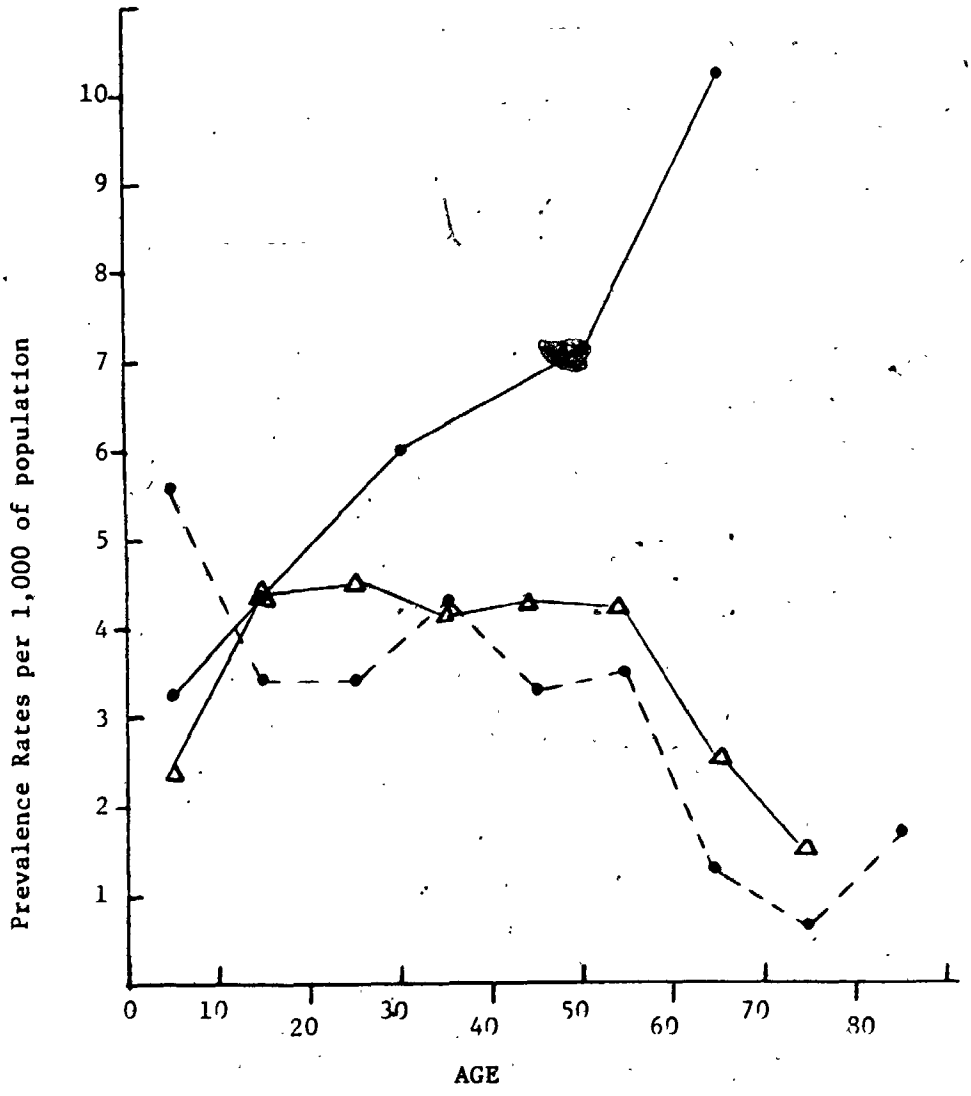
2.2.2 Prevalence Rates

The Kurland (1960) epidemiologic study of epilepsy utilized extensive data held at the Mayo Clinic. The clinic was then the only facility with diagnostic capacity in the area. Its neurologic records could then be regarded as relatively complete. Kurland included in his study any subject exhibiting more than one seizure, as long as it had not been the result of cardiovascular or metabolic origin, or hysterical in nature. Children with febrile convulsions were included, but dealt with separately in analysis. Twelve per cent of the subjects included had only exhibited one seizure, while 30% had only experienced febrile convulsions.

The age-specific prevalence rates adjusted to the U.S. population for 1950 appear in figure 1. A prevalence rate of 5.79 per 1,000 of the population was recorded in the first decade of life. This rate decreased dramatically to 3.45 per 1,000 during the second decade and remained generally stable until the sixth decade of life, when a further drop in rates occurred. Prevalence rates increased again for persons past their eightieth birthday.

Gudmundsson (1966) and Brewis et al. (1966) published epidemiologic

Prevalence of Epilepsy by Age



From Hauser and Kurland (1975). (1-1-1965 data only)

From Kurland (1960).

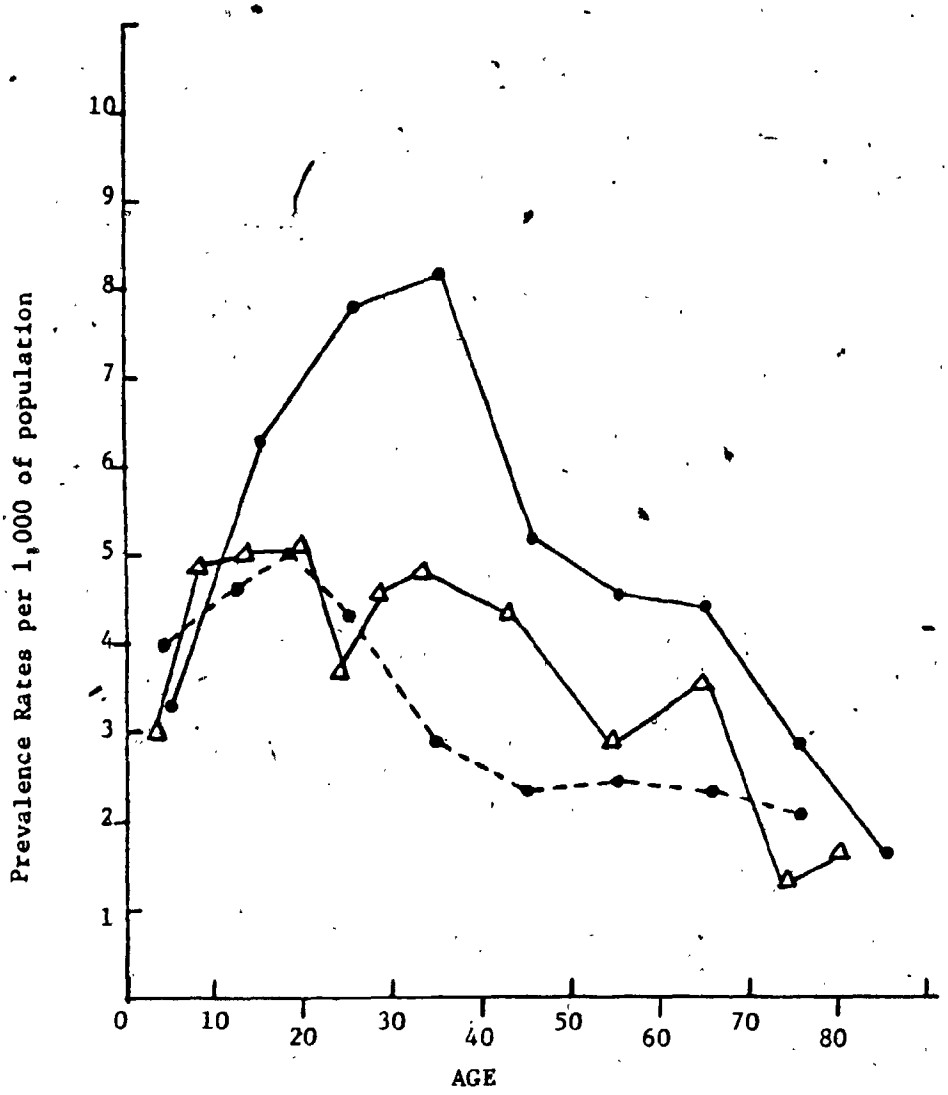
From Gudmundsson (1966).

(Figure 1)

studies of epilepsy in the same year. While Gudmundsson studied the population of Iceland, Brewis et al. examined the population of Carlisle. Both studies made extensive use of hospital records, general and private physician records and social welfare agency data. In addition, Brewis et al. (1966) included a household survey. Eleven point five per cent of all private household heads were contacted and a questionnaire on each resident family member completed. Prevalence rates for the Gudmundsson (1966) study are presented (see figure 1), while those of the Brewis et al. (1966) study are presented in figure 2. Both studies reported substantially lower rates (for their 0-9 year of age cohort) than the Kurland (1960) study. Gudmundsson (1966) reported a 2.42 per 1,000 rate, while Brewis et al. (1966) reported a 3.3 per 1,000 rate. These rates nearly doubled for the next category (10-19 years). While the rates reported by Brewis et al. (1966) continue to rise over the next two decades, those reported by Gudmundsson show remarkable stability until age sixty, when a marked decrease in rates occurs. Both studies then, like the Kurland (1960) study, show falling prevalence rates in later life, starting around the latter part of the fifth decade.

Leibowtz and Alter (1968) utilised the diagnostic records held at the department of neurology of the Hadassah-Hebrew University Hospital (the only such facility in Jerusalem at the time) to examine rates of epilepsy in the population of Jerusalem. This study was based solely on the examination of diagnostic records, with attendant potential biases. In spite of this, the Leibowtz and Alter (1968) study reported significantly higher rates of epilepsy than a similar study on the same population, published just a year earlier (Wajsbort et al. 1967).

Prevalence of Epilepsy by Age



From Brewis et al. (1966).

From de Graaf (1974).

From Leibowtz and Alter (1968).

(Figure 2)

Prevalence rates for the Leibowtz and Alter study are presented in figure 2. Rates were categorised in shorter age brackets than previous studies. However, visual comparison of graphs presented in figures 1 and 2 suggests very little difference in trends between the Leibowtz and Alter (1968) study , and those of Kurland (1960) and Gudmundsson (1966). The magnitude of reported rates between these studies varies considerably. Leibowtz and Alter (1968) reported a mean prevalence rate of 3 per 1,000 of the population for their 0-4 years old cohort. This rate increased to 4.8 per 1,000 in the 5-9 years old cohort and remained substantially stable up to the 35-44 years of age bracket. After this, an essentially downward trend in rates was reported.

The de Graaf (1974) study varies significantly from other previously reported studies. A prevalence rate of 4 per 1,000 reported in the 0-6 years of age bracket increased to 5 per 1,000 in the 15-19 years old cohort; only to undergo a steady downward trend for all other ensuing age brackets (see figure 2). This study relied again on diagnostic records compiled at a local neurological department over a five year period (1968-1972). Although children with a diagnosis of febrile convulsions, as well as others exhibiting a normal E.E.G., were excluded, the author reported that children were rarely examined neurologically. This is perplexing in light of rates reported for the 0-6 years old group which are higher than any other preceding rates reported here, with the exception of the Kurland (1960) rates. de Graaf (1974) further reported that his population lived in sub-Arctic Norway; for many members of this population, a visit to a medical clinic entailed some two days of travel. Thus steadily declining rates reported after the second decade of life in this study may be attributed to poor case

ascertainment, high mortality or a combination of both. Accepting the above brings the rates reported by de Graaf for his 15-19 years of age cohort into sharper focus. This rate (5 per 1,000 of the population) is very similar to rates for the same age brackets reported by Gudmundsson (1966), Brewis et al. (1966), and Leibowtz and Alter (1968). The Kurland (1960) study reported lower rates for this age bracket.

Hauser and Kurland (1975) reported the prevalence of epilepsy in Rochester, Minnesota between 1935 and 1967. Their case ascertainment and subject inclusion criteria were probably better than those of any other epilepsy study. In spite of this, these authors expressed concern about the possibility of underestimation of rates. Transitory remissions, relocation of qualifying subjects (both in and out of the community), and the exclusion of epileptics not diagnosed during the study period may have contributed to under-representation. In addition, although these authors reported rates standardised to U.S. census figures, blacks and Hispanics, not represented in the Hauser and Kurland (1975) data, were included in the census figures. As will be shown later, these races suffer significantly higher rates of epilepsy than whites. Only 1965 point-prevalence rates are presented (see figure 1) in this study, since they were identified by the authors as potentially the best in terms of case ascertainment. Rates reported by the Hauser and Kurland (1975) study are decidedly incremental in trend; low in the first decade of life (3.3 per 1,000 of the population), they increase with advancing age to 10.2 per 1,000 in the sixty and over cohort.

Hauser and Kurland (1975) also published age-specific point-prevalence rates for 1940, 1950 and 1960. Although rates for the first two age brackets in each of these years vary over time, no trend

emerged. The same was not true of the remaining age brackets; increases in rates over time were reported. This may have been due in part to better case ascertainment, or an actual increase in rates as a result of an altered life style. The remarkable upward trend over time for the 60 and over cohort (from 3.4 per 1,000 in 1940 to 10.2 per 1,000 in 1965) may have been due to better case ascertainment, higher morbidity, and lower mortality as a result of better medical management, leading to a synergistic cohort effect.

Gudmundsson (1966) reported male preponderance in prevalence rates for his 0-9, 30-39, and 70+ age brackets. All other age categories showed female predominance in rates. The 1967 Wajsbort et al. data showed no significant sex-specific differences in prevalence rates until the fourth decade of life, when rates for males were significantly higher than those of females. The de Graaf (1974) study showed clear female predominance in rates in the 15-19 years of age bracket. Although male prevalence rates were consistently higher than those of females in all four study periods, Hauser and Kurland (1975) reported that these differences were not statistically significant. Inclusion of black and Hispanic subjects (had they been available for the study) may have altered the picture considerably. Other studies discussed previously failed to categorise their data by sex.

2.2.2.1 Prevalence Rates Overview

A large number of other epidemiologic studies of epilepsy have been carried out. They have not been included here because of various inherent deficiencies, making comparison meaningless or impossible. However, the six studies previously discussed are sufficient to illustrate the many problems involved in studying this

disorder. The Hauser and Kurland (1975) study reported significantly higher prevalence rates than the Kurland (1960) study. Both studies were carried out in the same community using very similar case ascertainment and subject admission criteria. Hauser and Kurland (1975) suggested that this discrepancy may have been attributable to more intensive examination of records (in their 1975 study), and an actual increase in rates resultant from a re-examination of previously excluded "mis-diagnosed/vaguely reported" prospective subjects' records.

The highest prevalence rates for epilepsy in the Kurland (1960) study were reported for the first decade of life. Thereafter, rates are generally lower and stable. These rates are diametrically opposite to those reported by the Hauser and Kurland (1975) study. Indeed, prevalence rates reported in this later Rochester study are in general higher than those of any others examined here. The lengthy period of study, in association with lower mortality rates and improved case ascertainment in the aged, may have contributed to these higher rates.

Comparison between the other studies previously examined points to an increase in rates during the second decade of life. This is generally followed by stability with minor fluctuations until around the fifth decade of life, when a significant decrement in rates occurs. Earlier reductions in rates (around age 30), reported by de Graaf (1974), may be solely due to the difficulty encountered by prospective subjects in reaching diagnostic centres, in sparsely populated Arctic regions.

As pointed out earlier, sex-specific rates vary, with higher rates for females reported by Gudmundsson (1966) in the 10-29 years of age cohort, and de Graaf (1974) in his 15-19 years of age group.

2.2.3 Incidence Rates

Males tend to suffer from epilepsy more often than females, although some studies consider this difference in their reported incidence rates statistically non-significant. Turner (1907) reported a 1.3 to 1 male to female ratio; Kurland (1960), 1.9 to 1; Krohn (1961), 1.3 to 1; Gudmundsson (1966), 1.2 to 1; Wajsbort (1967), 1.3 to 1; Leibowtz and Alter (1968), 1.3 to 1; Hauser and Kurland (1975), 1.2 to 1; Juul-Jensen and Foldspang (1983), 1.4 to 1; Granieri et al. (1983), 1.4 to 1. Not all studies reported a male predominance in incidence rates however. Pond et al. (1960) and Goodridge and Shorvon (1983) both reported female preponderance. Leviton and Cowan (1983) proposed that:

> "Differences in reported sex ratio of epilepsy incidence rates may reflect differences in the age groups studied, differences in the distribution of seizure types, geographic variations in exposure risk factors because of cultural or social differences in the rearing of male and female children, methodological differences in case identification or variation in diagnosis by sex."

A bi-modal, age-specific distribution of incidence rates of epilepsy was described by Kurland (1960), Crombie et al. (1960), Brewis et al. (1966), Gudmundsson (1966), Wajsbort et al. (1967), van den Berg and Yerushalmy (1969), de Graaf (1974) and Hauser and Kurland (1975). Although not all studies reported rates specific to the first year of life, those that did confirmed that the highest rates of epilepsy occurred at this time of life: Kurland (1960), Gudmundsson (1966), van den Berg and Yerushalmy (1969), Gomez (1972), Gates (1972), Shamanski and Glaser (1979), Hauser and Kurland (1975), Leviton and Cowan (1982), Juul-Jensen and Foldspang (1983), Granieri et al. (1983). In addition, both Gomez (1972) and Gates (1972) reported that in the first year of life, risk of developing seizures was highest in the first month of life.

A female predominance in rates of epilepsy during the first year of life was reported by Wajsbort et al. (1967), Goodridge and Shorvon (1983). In general, however, reported rates were consistently higher for males.

Incidence rates fall dramatically after this first year (see figures 3 to 5). Between the later part of the first decade of life and the first half of the second decade, changes in rates have been reported, pointing to a slowdown in the rate of decrement.

Loiseau et al. (1983) reported that although epilepsy of adolescence accounts for between 21.3% (Leibowtz and Alter, 1968) and 38% (Juul-Jensen, 1964) of all epilepsies, little had been written about it. This phenomenon was in fact noted as early as 1907 by Turner who stated:

> ".. a small decline [in rates] then takes place about the eighth or ninth years, preliminary to the steady increase in the incidence of this disease which reaches a maximum between the ages of twelve and fifteen."

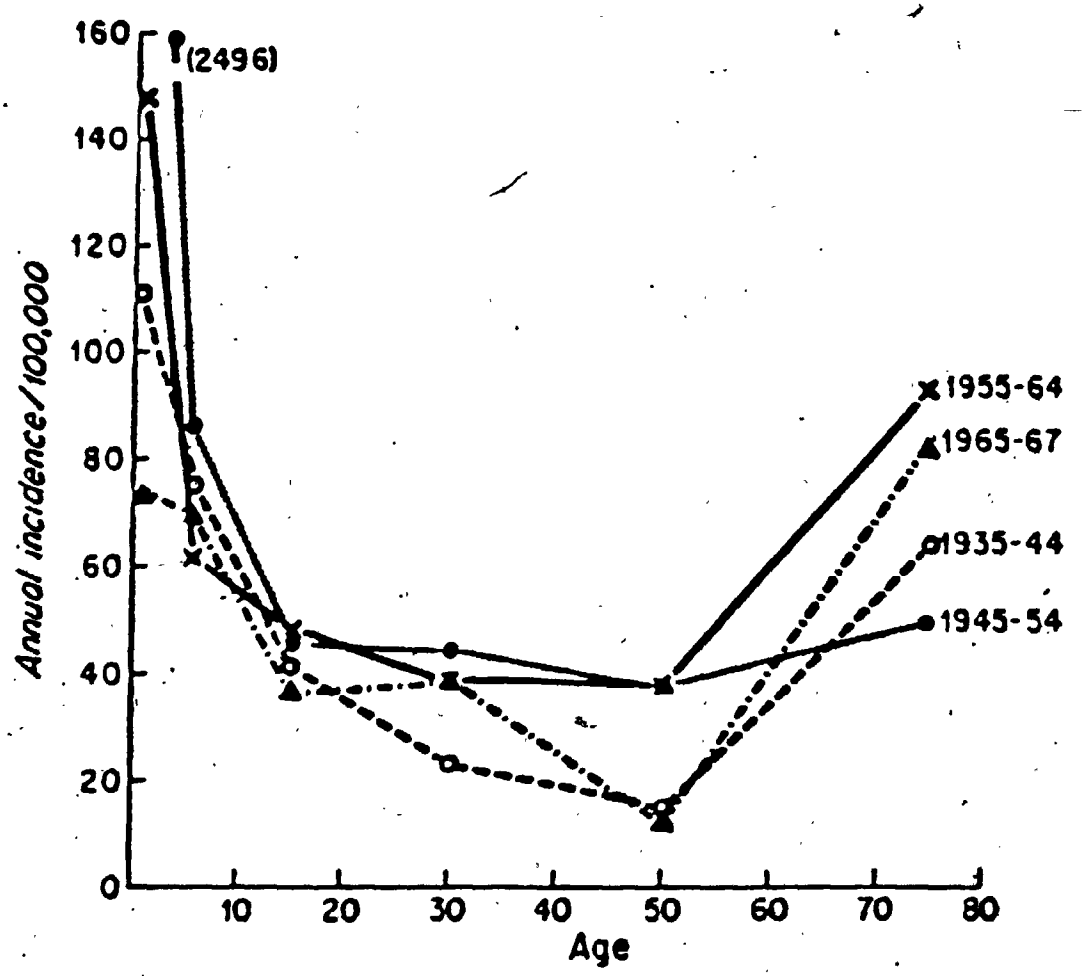
More recent studies have confirmed the clear changes in rates taking place around this age bracket (see figure 3).

What is however more important, is the fact that these changes in rates are female-specific (see figures 4 and 5). Kurland (1960) did not report any increments in incidence rates around this age bracket, however using the updated data of Hauser and Kurland (1975) for the same population and study period (as used by Kurland, 1960), it is obvious that the angle of the slope is different for females than it is for males (see figure 4).

Brewis et al. (1966) demonstrated an increase in incidence rates between the ages of ten and nineteen. Following a substantial decline in rates (from 75.3 per 100,000 for the 0-4 years of age cohort, to

Mean Annual Incidence of Epilepsy by Age and Study Period

Rochester, Minnesota. 1935-67.

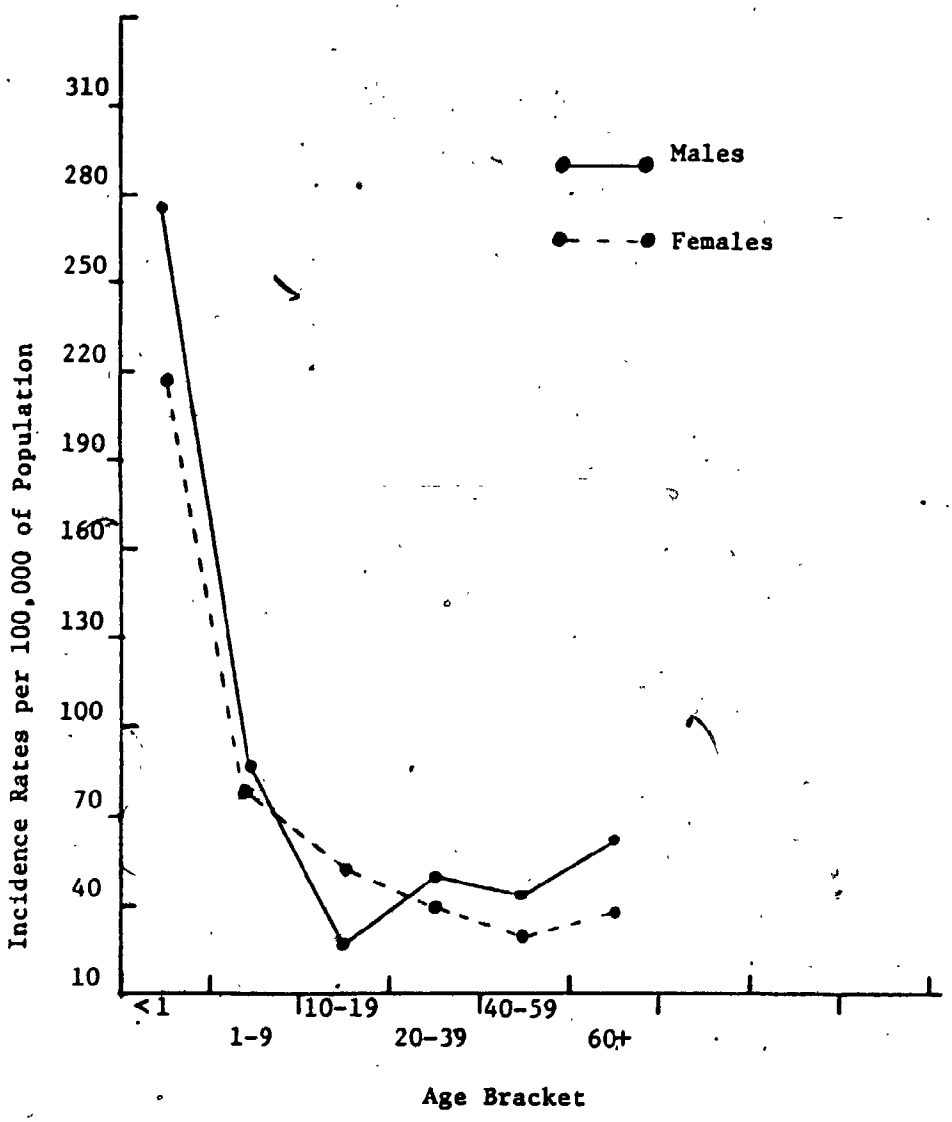


From: Hauser and Kurland (1975)

(Figure 3)

Incidence of Epilepsy by Age and Sex

Rochester Minnesota, 1945 - 1954

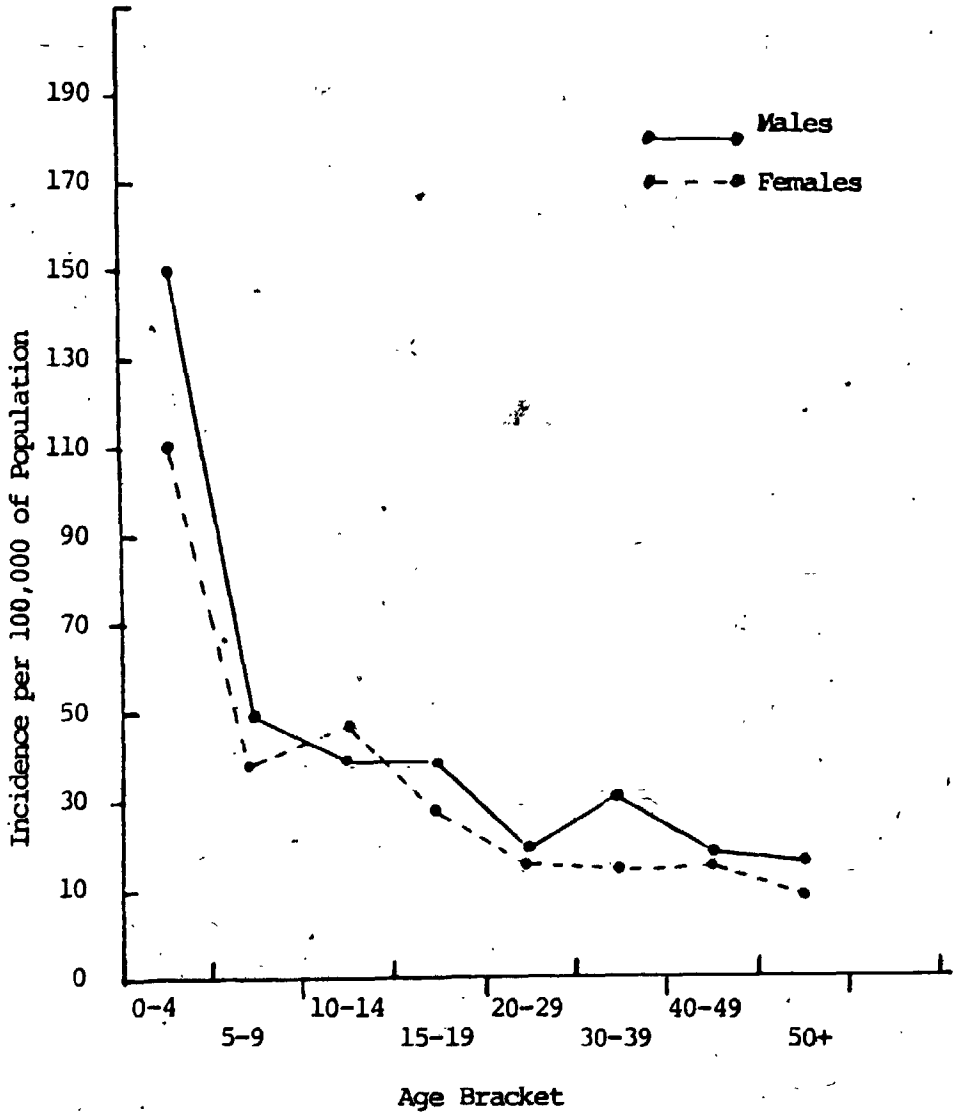


From: Hauser and Kurland (1975)

(Figure 4)

Mean Incidence Rates of Epilepsy by Age and Sex

Aarhus, Denmark 1967-1977



From: Juul-Jensen and Foldspang (1983)

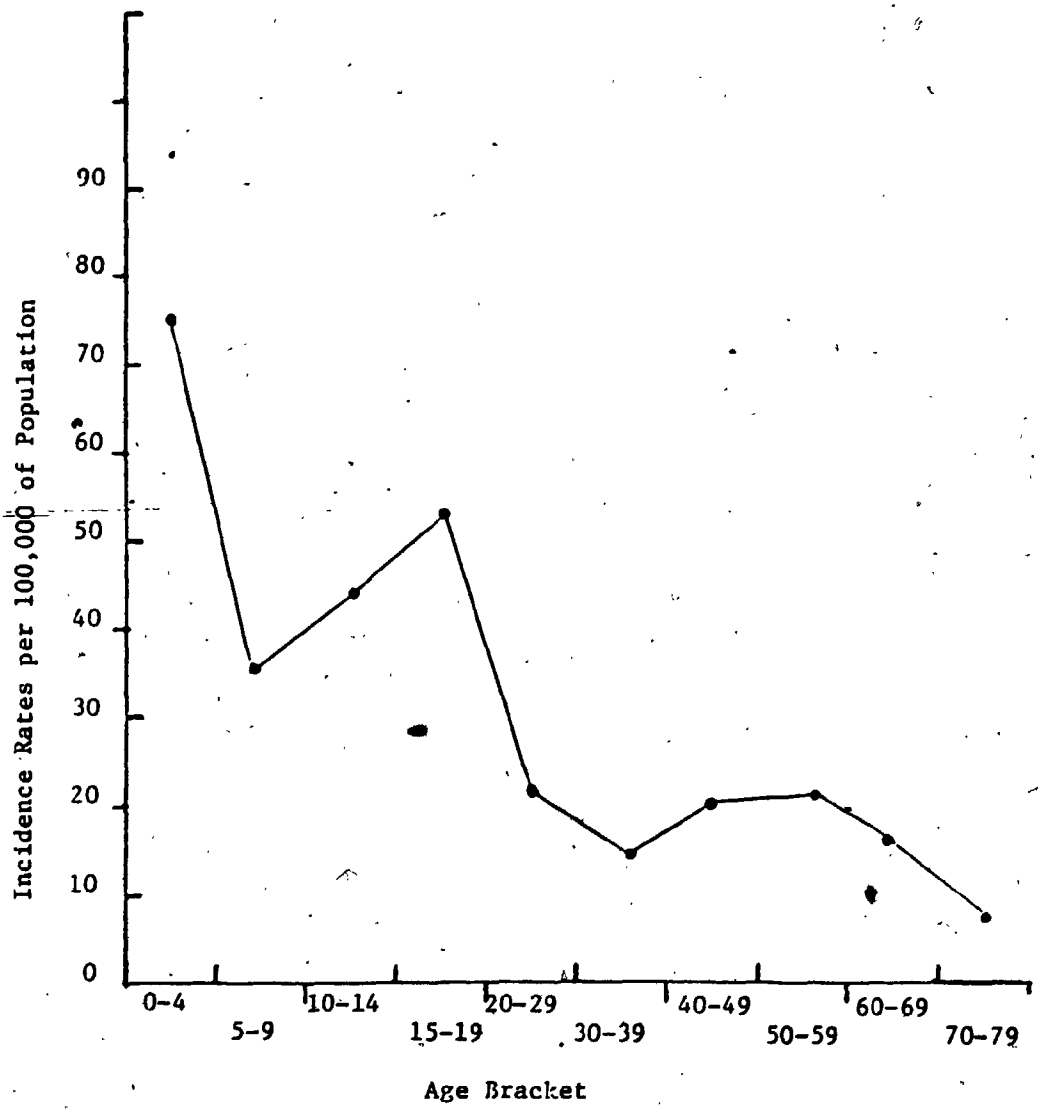
(Figure 5)

36.0 per 100,000 for the 5-9 years of age group), incidence rates for the 10-14 years of age bracket climbed to 44.3 and to 53.4 per 100,000 in the 15-19 years of age cohort. A generally stable lower incidence rate ensues to the seventh decade of life, when a further drop in rates is reported (see figure 6). Hughes (1967) carried out a retrospective analysis of electroencephalographic (E.E.G.) records and demonstrated a clear peak in incidence rates (abnormal recordings clearly indicative of a convulsive disorder) at age fifteen. Once again, sex-specific differences were not reported. Although the data of Hughes continued to confirm changes in rates at adolescence, it must nonetheless be treated with caution. E.E.G. recordings deemed indicative of a seizure disorder were not, however, backed by a clear history of convulsive activity. While a normal E.E.G. does not preclude a seizure disorder, an abnormal E.E.G. recording is not necessarily indicative of a seizure disorder (Sofijanov, 1982).

Blom et al. (1978) examined children three years after presumed onset of their epilepsy. They reported an incidence rate of 71 per 100,000 in their 10-15 years of age cohort, an increase of 19 per 100,000 over the rates of the previous age bracket. However, no sex-specific rates were reported. In contrast, the data reported by Shamanski and Glaser (1979) are presented by age, sex and racial categories. The incidence of epilepsy in this study is reported by yearly interval for the first fourteen years of life. Thus, although no direct comparison with other studies is possible (due to different data categorisation and the limited age-specific nature of the material), its focus on childhood and adolescent epilepsy made it invaluable to the topic under examination here.

Incidence of Epilepsy by Age

Carlisle 1955-1961



From: Brewis et al. (1966)

(Figure 6)

Race-specific data in the Shamansky and Glaser (1979) study is subdivided into two categories - Whites and Blacks. The "Blacks" category includes in it members of Hispanic ethnicity, on the premise that their socio-economic status approximates that of blacks more readily than it does that of whites. White and black male incidence rates show the classic high incidence rates in the first year of life with diminishing rates thereafter. An upward trend in rates is discernable around the thirteenth year of life. Black male rates are in general higher than those of white males (see Table 1). Female rates of epilepsy for both whites and blacks were also high in the first year of life, then diminished with increasing age. However, while rates of white females continued to decline up to the thirteenth year of life, rates of epilepsy for black females increase substantially starting around the ninth year of life. More importantly, reported rates for white females were higher than those of males (between the ages of 9 and 10). The same applied to black females although the magnitude of the difference in rates was bigger.

A certain degree of caution must be exercised in the interpretation of these rates, since the potential sources of bias were numerous. It can however be noted that sex-specific differences in rates reported in previous studies were once again confirmed. In addition, race-specific differences were highly noticeable, and confirmed the previous observations of Gates (1972).

Leviton and Cowan (1982) reviewed previous epidemiologic studies focusing on childhood epilepsy. They concluded that higher incidence rates of epilepsy were found in males before ten years of age, and in females during adolescence. They further identified the data of Crombie

"Incidence of Epilepsy in the New Haven Area (1960 - 1970)

Annual Incidence Rates per 100,000 by Age, Sex and Race

	MALE		FEMALE	
Age	White	Black*	White	Black*
<1	143.0	263.0	101.5	180.0
1	82.5	133.0	106.0	195.0
2	84.5	282.5	55.0	180.0
3	56.5	431.5	74.0	82.5
4	85.0	269.5	55.5	112.5
5	65.0	104.5	47.5	71.5
6	56.0	89.0	53.5	87.0
7	67.5	120.5	64.0	94.5
8	46.5	104.5	56.0	16.0
9	51.0	31.5	53.0	44.0
10	38.0	60.0	67.0	73.0
11	35.5	108.5	40.5	147.0
12	27.0	37.5	35.0	169.0
13	45.5	18.5	38.5	129.5
14	62.5	220.0	63.5	93.5

*Category includes subjects of Hispanic ethnicity,

From : Shamanski and Glaser (1979)

Table 1.

et al. (1960) as exceptional in its reported increase in rates starting around age seven, and continuing to the last reported age bracket.

Goodridge and Shorvon (1982) were unable to offer any explanation for female predominance in incidence rates of epilepsy, reported in their first three age brackets (1-30 years of age). Similar findings had been reported by only one previous study - the already discussed study of Pond et al. (1960).

The epidemiological data presented in the Hauser and Kurland (1975) study has so far been left out of the chronological sequence for a number of reasons. As has been already noted, it is not only acknowledged as a bench mark in the epidemiology of epilepsy, but more importantly the wealth of data presented merits exclusive examination.

Incidence rates for epilepsy over the whole study period (1935-1967) were reported in conjunction with age, sex, seizure type, and aetiology-specific rates. No sex-specific statistically significant differences were found either in the individual decennial categories presented, or in the study as a whole when all types of epilepsy were grouped together. Rates were generally high in the first year of life and decreased up to around the sixth decade of life, when further elevations in rates were reported again (see figure 3). What is however apparent from visual inspection of figure 3 is the change in angle of the slope around the middle of the second decade of life. Thus once again, this study confirms previously discussed changes in incidence rates at this time period.

Hauser and Kurland (1975) reported that approximately 75% of their epileptic subjects had epilepsy of unknown aetiology. This group is of particular importance to this study. Male predominance in incidence

rates of epilepsy for this group continued to be reported for all ages except the 10-19 years of age bracket. Hauser and Kurland (1975) reported that:

> "At ages 10 to 19 in each time interval females had higher incidence rates of idiopathic epilepsy than did males, but the difference was significant only in the 1945-1954 interval."

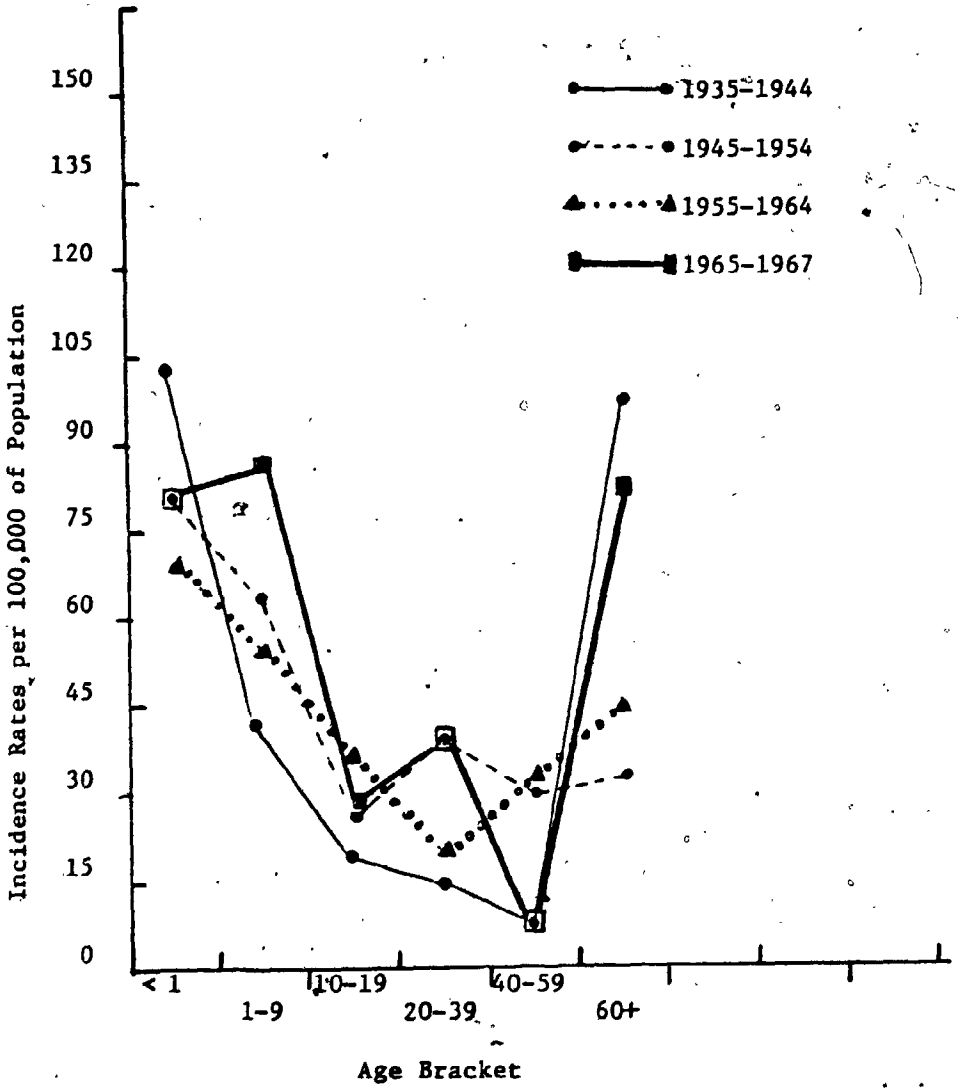
Visual inspection of figures 7 and 8 readily portrays the difference in slopes for males and females in each of the four time periods presented. A number of reasons, previously discussed, could account for the statistical non-significance of sex differences reported in the other three time periods. What is however more relevant is the fact that sex-specific differences in rates reported by several other studies, using a number of different study inclusion criteria, were once again confirmed by the Hauser and Kurland (1975) study. Thus these changes in rates can not be attributed to chance alone.

2.2.3.1 Epilepsy Incidence Rates Overview

Epilepsy then appears to affect more males than females, although the difference in risk is at best conjectural. Highest rates of this disorder are registered in the first year of life, and then tend to decline with increasing age. Males tend to predominate in rates for approximately the first ten years of life, while females tend to have a higher risk for the disorder during adolescence. An alteration in incidence rate slope angle has been observed by several researchers. In general this alteration in slope pointed to a less rapid decrease in rates. Corsellis (1974) discussed the maturational theories of Gastaut (1959) and Veith (1970). These researchers proposed that a variety of cerebral insults occasioned at or shortly after birth matured at a later

Male Incidence Rates of Epilepsy (Idiopathic)

By Age and Study Period

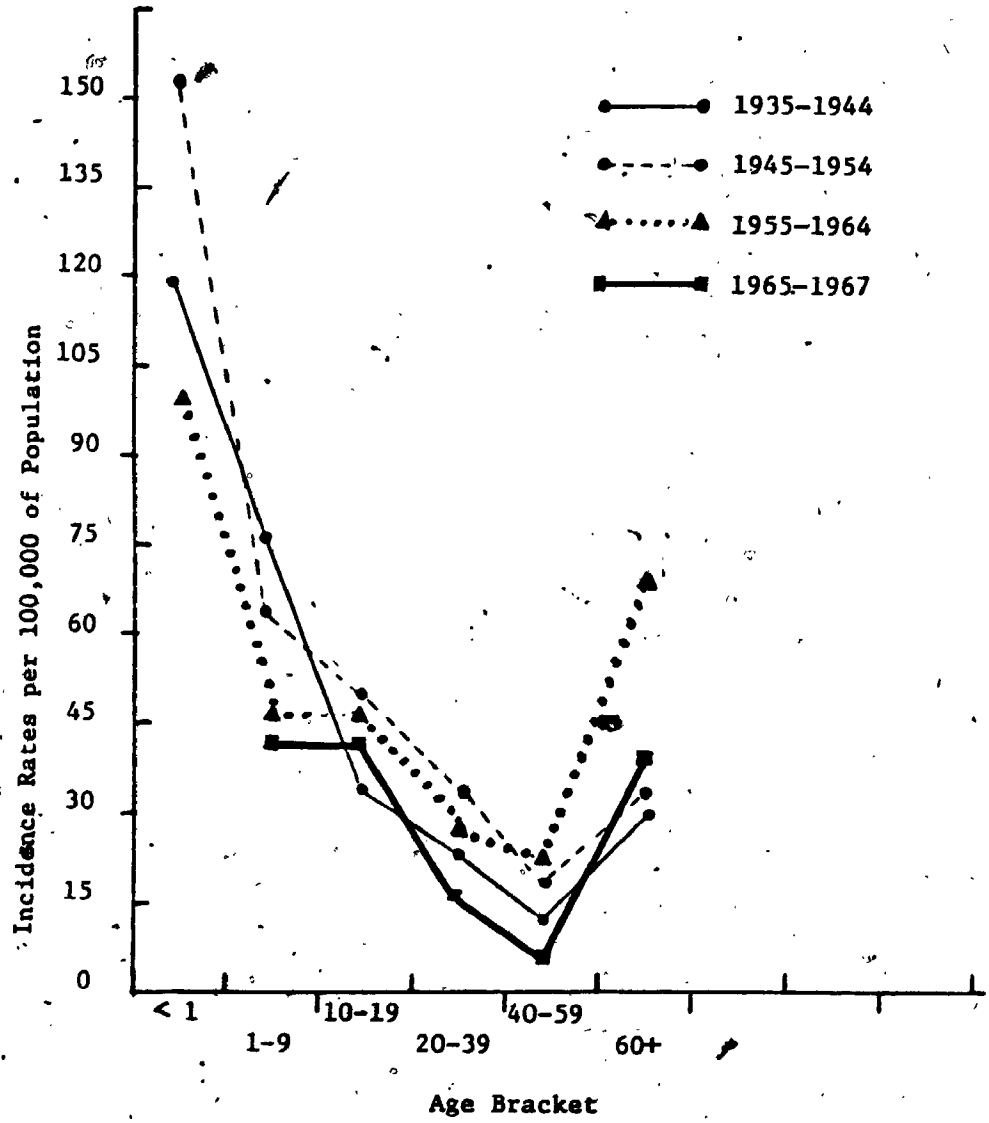


From: Hauser and Kurland (1975)

(Figure 7)

Female Incidence Rates of Epilepsy (Idiopathic)

By Age and Study Period



From: Hauser and Kurland (1975)

(Figure 8)

date into active epileptic foci. In addition, an age-dependent risk for head trauma has been consistently reported for these adolescent cohorts. As will be shown, this risk of head trauma is also highly sex-specific. After adolescence, incidence rates of epilepsy once again decrease and continue to do so into later life, although some studies have reported small increases beginning about the sixth decade of life.

The rates of epilepsy during adolescence/puberty appear to undergo transitory changes which are not congruent with the general trends of rates of the disorder.

2.3 Epilepsy in Adolescence

Regardless of ethnicity, race or geographic location, adolescence has been associated with increasing social independence. Correlated with this independence is an increased risk for injury - head trauma in particular: Paillas et al. (1970), Klonoff and Robinson (1967), Klonoff and Thompson (1969), Kraus et al. (1975), Kraus (1978). A number of prospective studies reported the incidence of epilepsy following head injury: Phillips (1954), Caveness and Liss (1961), Kerr et al. (1971), Kraus (1978, 1980). This risk ranged from approximately 40% of all closed head injury (Jennett, 1973) to 25% when depressed skull fracture (without rupture of Dura) occurred, (Jennett, 1974). The predominant feature of these epidemiologic studies however was male predominance in rates, from a high of 81% (Kerr et al., 1971) to a modest 59% (Kraus, 1978). The increase of incidence rates of epilepsy in the female around adolescence can not be accounted for by increases in head injury reported at this time. On the contrary, the rise in female incidence rates of epilepsy is

brought into sharper focus by these head trauma statistics.

As discussed previously, a "maturational" theory has existed for some time. Although primarily presented as a hypothesis for temporal lobe epilepsy, this theory was later utilised as a model for other forms of epilepsy as well. The work of Gastaut (1959) and Meyer et al. (1954), however, clearly demonstrated the great complexity of the pathogenic mechanisms underlying seizure disorder (Corsellis, 1974). It is then highly unlikely that previously discussed changes in rates around puberty could be accounted for by this maturational theory.

The single most important event of this age bracket is the onset of physical and reproductive maturation. A positive association between puberty and epilepsy has been reported: Sieveking (1857), Gowers (1885), Turner (1907), Rees (1953), Ansell and Clarke (1956), Jeavons (1977), Jeavons et al. (1977), Newmark and Penry (1980), Rosciszewska (1980).

2.4 Hormonal Correlates of Puberty

Both sexes undergo this maturational process, although in general the female matures some two years earlier than the male (Tanner, 1981). Onset of reproductive maturation is controlled to some degree genetically, socio-culturally and environmentally. In the final analysis, however, hormonal interactions in the hypothalamo-pituitary-gonadal axis are the prime motivators.

Sexual maturation commences at the the later part of the musculo-skeletal growth spurt, with increasing release of Luteinizing-Hormone-Releasing-Hormone (L.H.R.H.) from the hypothalamus. This stimulates the production of pituitary gonadotropins Follicle-Stimulating Hormone (F.S.H.), and Luteinizing Hormone (L.H.), (Tanner, 1981; Gruber and Lucas, 1976). These gonadotropins in turn stimulate increasing production

of the two basic sex-steroids - Testosterone for the male and Estrogen for the female. Radical changes in the secreting/inhibitory feedback mechanisms allow for large increases in blood levels of these steroids. Once maturation and full reproductive potential is achieved some two years following onset, production of gonadotropins continues to be mediated by the same feedback system. However, while L.H. stimulates production of testosterone in a pulsatile fashion in the male, F.S.H. stimulates estrogen production in the female cyclically. Post-pubertal levels of both gonadotropins and sex-steroids are much higher than those recorded pre-pubertally, but the cyclical nature of steroid production in the female allows for easier intra-cycle comparison over a considerable period of time. Retrospective as well as prospective inferences may be then rather easier to make under these circumstances.

2.5 Physiology of the Menstrual Cycle

The menstrual cycle can be divided into three stages: the Follicular Stage, Ovulation, the Luteal Phase. Further sub-division of these stages is necessary to explain a highly complex phenomenon.

2.5.1 The Antral Stage

The follicular stage starts with the aromatization of androgens into estrogen by the action of Follicle Stimulating Hormone (F.S.H.) (Moon et al., 1975). Intra-follicular estrogen combines with F.S.H. to stimulate rapid proliferation of granulosa cells - promoting follicular growth: Richards (1979), Goldenberg et al. (1972). The appearance of nutritive follicular fluid (containing estrogen and F.S.H.) in the intercellular spaces of granulosa cells signals the start of the antral stage: McNatty et al. (1979). The follicle with the greatest granulosa cell proliferation, and highest estrogen content, is the most

likely to house a healthy oocyte and proceed to maturation (Fritz and Speroff, 1982). Follicular estrogen interacts with F.S.H. at the hypothalamo-hypophyseal level to withdraw gonadotropin support from other less well developed but competing follicles (Zeleznik, 1981). This process will, with rare exception, guarantee the ovulation of a single oocyte in every cycle. Elevated estrogen levels in the dominant follicle combine with a now diminishing F.S.H. to produce intra-follicular receptor sites for Luteinizing Hormone (L.H.), that will become increasingly important as ovulation approaches.

2.5.2 The Pre-Ovulatory Stage

As it approaches maturity, the dominant follicle produces increasing amounts of estrogen (E) that surges to a peak approximately 24 to 36 hours prior to ovulation (Pauerstein et al., 1978). F.S.H. shows concommitant decrease, reaching a nadir as E peaks. The sustained high levels of E also stimulate the production of L.H. (Djahanbakhch et al., 1984). Luteinization of the follicle results in the production of progesterone (P), as well as increasing pulsatile levels of L.H. (Fleming and Coutts, 1982).

2.5.3 Ovulation

As L.H. levels rise to a peak, the follicle synthesises prostaglandins (P.Gs.). Prostaglandins, especially P.G.E. and P.G.F., are involved through an as yet unknown mechanism in follicular rapture and ovulation (O'Grady et al., 1972). The L.H. peak, which directly precedes ovulation by some 10 hours (Garcia et al., 1981), occurs as E levels plunge to a nadir.

2.5.4 The Luteal Phase

Following ovulation, the follicular granulosa cells enlarge

and accumulate lutein. thus becoming part of the Corpus Luteum (C.L.). The C.L. is responsible for P production in the luteal phase (di Zerega and Hodgen, 1980). Progesterone levels rise sharply after ovulation, reaching a peak some 8 days after the L.H. peak in the mid-luteal phase (Fritz and Speroff, 1982). The duration of progesterone production, and thus the viability of the corpus luteum, is highly dependent on continued L.H. stimulation as well as the sensitivity of the C.L. to L.H. However P production declines as E rises again mid-luteally. Butler et al. (1975) and Karsch et al. (1973) suggested that estrogen may also be involved (with L.H.) in a luteolytic action. As P falls to a nadir, menses occurs.

2.6 Effects of Estrogen and Progesterone

2.6.1 Effects of Estrogen and Progesterone on Seizure Threshold

Estrogen, both in its systemic form or topically administered to the cortex, lowers seizure threshold (Logothetis et al., 1959; Woolley et al., 1961; Woolley and Timiras, 1962a, b; Marcus et al., 1966; Stitt and Kinnard, 1968; Terasawa and Timiras, 1969; Timiras, 1969; Marcus, 1972; Zimmermann et al., 1973; Steger, 1976). Laidlaw (1956) found decreased seizure activity during the mid-luteal phase of the menstrual cycle in humans and suggested that elevated levels of progesterone found in the female at this time of the cycle mitigated the convulsant action of estrogens. Similar observations on the convulsant action of estrogen and the anti-convulsant action of progesterone on seizure activity in the female were made by Sanchez Longo and Gonzalez Saldana (1966), Buntner and Rosciszewska (1975), Backstrom (1976), Striano et al. (1979) and Rosciszewska (1980).

2.6.2 Effects of Estrogen and Progesterone on Ionized Calcium

Estrogen lowers plasma ionized calcium levels by inhibiting bone resorption of calcium (Burnkhardt et al., 1975). This had also been previously reported by Young and Nordin (1967) and Aitken et al. (1973) in their studies with post-menopausal females. Eleil et al. (1971), Tan et al. (1972) and Pitkin and Gebhardt (1977) found the same results in their studies with females in their last trimester of pregnancy. In addition, Simpson and Dale (1972) and Gray et al. (1982) reported that females taking oral contraceptives containing estrogen exhibited lower plasma calcium levels than controls.

Pitkin et al. (1978) demonstrated that parathyroid hormone (P.T.H.) and estrogen both rose during the follicular stage of the menstrual cycle. Estrogen attains a secondary peak during the mid-luteal phase of the cycle. P.T.H. levels failed to show an increase at this time. Pitkin et al. (1978) attributed this phenomenon to the mitigating action of progesterone on estrogen. Thus when estrogen levels were elevated, serum ionized calcium levels decreased. In an attempt to correct these deficient ionized calcium levels, the production of P.T.H. is stimulated. In the presence of elevated levels of progesterone, P.T.H. levels fail to rise, even when estrogen levels are elevated. Pitkin et al. (1978) suggested that progesterone may mimic the action of P.T.H.

Baran et al. (1980) were unable to replicate the results of Pitkin et al. (1978). They suspected an inadequate data gathering technique may have been the reason for their failure. It appears from the foregoing that estrogen and progesterone play significant roles in calcium metabolism. However, Johnston and Epstein (1982) stated that their

mechanism of action remains unknown. Kannis et al. (1982) suggested that a close correlation between levels of estrogen and levels of 1,25-dihydroxycalciferol ($1,25(OH)_2D_3$) had been demonstrated by Howland and Kramer as early as 1921. Kannis et al. (1982) further proposed that estrogens may act on calcium metabolism, at least in part, through increased binding of 1,25-dihydroxycalciferol, rather than through an increase of free levels of this hormone. Habener and Jacobs (1982) cited a number of studies demonstrating conflicting results about the possible effects of 1,25-dihydroxycalciferol on parathyroid hormone. However, Henry and Norman (1975) showed that traces of a tritium-labelled dose of 1,25-dihydroxycalciferol administered to chicks were found in their parathyroid gland as well as in their gut and bones. Although no evidence for this transport exists to date in the human, this evidence may supply a further hypothesis for estrogenic action on calcium metabolism.

2.7 Other Factors Affecting Ionized Calcium Levels

The importance of Vitamin D metabolism to bone resorption of calcium has been demonstrated in vitro (Raisz et al., 1972; Stern et al., 1977), and to a lesser extent in the human (Marshall and Nordin, 1977; Russell et al., 1974). Even in the presence of elevated P.T.H. levels, calcium is poorly absorbed by the gastro-intestinal tract if vitamin D metabolism is deficient: Hertelendy et al. (1960), Rasmussen et al. (1963), Arnaud et al. (1966), Peacock (1976), David et al. (1983). Chronic ingestion of the anticonvulsants phenobarbitone, primidone and phenytoin sodium has been shown to disrupt vitamin D metabolism and absorption (Richens and Rowe, 1970; Dent et al., 1970; Bowden, 1974; Christiansen et al., 1974; Mosekilde et al., 1979; Bell

et al., 1979). Rowe and Harris (1976) further suggested that vitamin D deficiency may be only one of the factors behind the low calcium levels found in some epileptic subjects. Specimens incubated in media containing phenytoin sodium, diazepam and carbamazepine were shown by Rowe and Harris (1976) to resist calcium resorption even in the presence of an otherwise facilitative parathyroid hormone. The same culture medium with phenobarbitone added to it did not produce similar results. These findings were later confirmed by Stamp et al. (1976).

Aladjem et al. (1980) were unable to find any differences in calcium levels between epileptic subjects and controls. They postulated that renal-tubule re-absorption of calcium, prior to its excretion, compensated for deficient calcium absorption from the gastro-intestinal tract. They further suggested that lengthy exposure to sunlight (their subjects resided in Tel Aviv, Israel) and an adequate diet may have contributed to the absence of rickets in their experimental population. The hypotheses of Aladjem et al. (1980) are not congruent with results from other research. Although the role of ultraviolet photosynthesis is paramount to vitamin D pro-hormone production, Dent and Watson (1966) found clear evidence of osteoporosis in their epileptic subjects who were maintained on a low dose phenobarbitone regime but who otherwise led a normal existence. Knudson (1932) demonstrated the body's ability to synthesize and store large quantities of vitamin D pro-hormone in the limited sunshine months of summer and dispense it to the body after hydroxylation over the lengthy period of winter in temperate climates.

To argue then for a purely privational cause to hypocalcaemia in epilepsy is patently inadequate. What is apparent is the probable

multifactorial causality of abnormal calcium metabolism in the epileptic subject. The female epileptic presents further complicating factors through cyclic estrogen production with its attendant synergistic effects.

2.8 Effects of Ionized Calcium on Seizures

Bigwood (1924) first proposed the importance of ionized calcium levels to the generation of neuronal burst firing activity. Steinbach et al. (1944), Brink et al. (1946), Cole (1949) and Creese and Roberts (1955) demonstrated the stabilising effect of extra-cellular ionized calcium on the excitable cell. Conversely, low extra-cellular levels of ionized calcium increased excitation. On reviewing a number of previously published experimental results, Frankenhaeuser and Hodgkin (1957) stated that "... increasing external calcium concentration raises the threshold, increases the membrane resistance [to depolarization] and accelerates accomodation. Reducing the calcium concentration has converse effect ... ". Somjen and Kato (1968) found a dose-response relationship between magnesium, ionized calcium and neuronal response to electrical stimulation. The addition of either magnesium or ionized calcium to the microenvironment of a burst firing neurone depressed the firing. They further confirmed this depressant action of magnesium and ionized calcium a year later (Kato and Somjen, 1969). Kelly et al. (1969) and Krnjevic and Lisiewcza (1972) added that an elevation in magnesium and ionized calcium increased electrical threshold by interfering with voltage-dependent increases in the cell's permeability to sodium. Low ionized calcium was identified as a cause of epileptic seizures in neonatal hypocalcaemia and during renal failure (Gastaut et al., 1969). Corriol et al (1969) stated that the

findings of Gastaut et al. had been made earlier by a number of researchers, but had remained unpublished. Phillis et al. (1973) used Verapamil as an ionized calcium blocking agent to demonstrate the suppression of inhibitory actions on rat cerebro-cortical neurones by low ionized calcium levels.

Katzman (1981) demonstrated a cerebro-spinal fluid (C.S.F.) to plasma ionized calcium ratio of approximately 2 to 1 in the human. Zuckerman and Glaser (1973) found that seizure threshold rose in cats perfused with an artificial C.S.F. high in ionized calcium. Conversely, when the perfusate was lacking in ionized calcium, seizure threshold fell. Lux and Heinemann (1978) confirmed this relationship in the presence of small elevations of extracellular potassium and lowered extracellular ionized calcium levels. Dunwiddie and Lynch (1970) further added that even in the presence of normal potassium levels, a lowered ionized calcium level occasionally produced seizures. Benninger et al. (1980) produced spontaneous repetitive high voltage potentials resembling epileptic activity in guinea-pig hippocampal slices. These slices had been bathed in a medium similar to that found during electrical stimulation.

Finally, Trams and Lauter (1978) pointed out that the role of glia in epilepsy had been underestimated. These researchers suggested that glia might limit neuronal excitability by "... limiting extracellular build up of substances which might trigger synaptic transmission". While Trams and Lauter hypothesized that a defective ionized calcium-ATPase deficiency might allow an extracellular increment in excitatory substances, they were unsure whether the defective ionized calcium-ATPase system was the result or the cause of burst firing activity.

Woodbury et al. (1984) reported that chronic administration of phenytoin caused increased glial cell function.

2.9 Epileptogenesis

A unitary model of epilepsy does not exist. This not only reflects the complexity of the subject matter, but also emphasizes the great diversity of neural processes at different sites within the nervous system (Roberts, 1984). A number of animal models of focal epilepsy using a variety of convulsant agents have been published: Westrum et al., 1964; Dichter and Spencer, 1969 (I) and (II); Ward, 1969; Jasper, 1972; Ward, 1972; Harris, 1975; Prince, 1975; Prince et al., 1983.

Schwartzkroin and Wyler (1980) utilized the works of Ayala et al. (1973) and Atkinson and Ward (1964) in attempting to construct a unitary model of focal epilepsy. Using Hughlings Jackson's (1931) description of epileptiform activity (as resultant from excessive discharges from over-excited neurons), Schwartzkroin and Wyler and Prince and Connors (1984) proposed that every neuron possessed a propensity to burst firing. While some neurons (termed abnormal by Atkinson and Ward) exhibited burst firing spontaneously, others (as postulated by Ayala et al.) required exogenous influences to trigger this activity. Burst firing, Schwartzkroin and Wyler suggested, are triggered by essentially the same mechanisms that generate action potentials in other neurons. Finally, they reaffirmed the importance of the ionic environment. Small quantitative variations in the composition of this milieu could cause neurons to generate bursts of action potentials. This was previously confirmed by Somjem and Kato, 1968; Kelly et al., 1969; Krnjevic and Lisiewcza, 1972; Phillis et al., 1973; Roberts, 1984; Trams and Lauter, 1984; Woodbury et al., 1984.

Prince et al. (1984) confirmed the ability of some convulsants to change extracellular field potentials as well as intracellular activity in neuronal colonies. They further stated that while Penicillin did not alter resting membrane potentials, it did antagonise presumed GABA-mediated inhibitory processes. Similar findings were reported by Hill and Simmons, 1976; Wong and Prince, 1979; Dingledine and Gjerstad, 1980; Schwartzkroin and Prince, 1980; Prince et al., 1983. Thus, although not all neurons are capable of spontaneous depolarizing shifts, the absence of inhibitory post-synaptic potentials (I.P.S.Ps.) facilitated bursts of slow spikes, and generation of excitatory post-synaptic potentials (E.P.S.Ps.). Like many researchers before them, Prince et al. (1983) confirmed the excitatory effects of changes in the ionic milieu rendering neuronal populations more vulnerable to bursts of action potentials. In addition, they added that axonal terminals were capable of generating bursts of action potentials when exposed to microenvironmental imbalance.

Recently, increasing attention has been focused on the role of Calmodulin in neuronal excitability. The effects of ionized calcium on neuronal transmission are dependent only on the entry of ionized calcium into the nerve terminal. This runs counter to previous suspicion of dependence on presynaptic potentials (Miledi and Slate, 1966; Katz and Miledi, 1969, 1970; Miledi, 1973). From the foregoing, de Lorenzo (1984) hypothesized that as ionized calcium entered the presynaptic nerve terminal, it binded to calmodulin and activated several excitatory Ca^{++}-calmodulin regulated processes. Thus rapid (both local as well as systemic) changes in levels of ionized calcium may produce ionic milieu dysequilibrium, enhancing action potential proliferation and releasing

intracellular ionized calcium. Ca^{++} - calmodulin binding may generate and spread further bursts of action potentials in surrounding colonies of neurons.

Roberts (1984) stated: "The impression is gained that one is looking at a highly restrained nervous system, with the inhibitory neurons acting like reins that serve to keep neuronal 'horses' from running away." In the presence of an endogenous convulsant (estrogen), increased neuronal activity to produce this and other hormones associated with the female menstrual cycle, and microenvironmental changes, may significantly antagonise I.P.S.Ps. Intracellular (mitochondrial) release of ionized calcium can occur leading to quantal neuro-transmitter release (Chance, 1965; Katz, 1973; Alnaes et al., 1974; Rahamimoff et al., 1975). Generation of intense synchronised depolarizations leading to ictal discharges is not inconceivable.

2.10 Summary

The preceding review supplied the necessary information to formulate hypotheses. Estrogen has been used as a convulsant agent in seizure experimentation. It is found in fluctuating levels systematically, and is known to play a significant role in activating release of other hormones involved in the female menstrual cycle. Much of its action in this process is carried out directly on neural tissue. In addition, studies on post-menopausal females proposed and later confirmed its antagonistic action to calcium. The Pitkin et al. (1978) study further confirmed the action of estrogen on ionized calcium during the menstrual cycle.

Various studies have proposed and confirmed the importance of ionized action to neuronal function. While ionized calcium is necessary

for neuro-transmission, decreased levels of this mineral have also been associated with increased neuronal excitation, a lowering of seizure threshold and significant reduction in the production of I.P.S.Ps. An environment conducive to the generation of burst firing activity and its spread exists at least in theory during the menstrual cycle.

What must be born in mind, however, is the fact that a large percentage of the preceding reports and models were based on animal tissue experimentation, sometimes at the unicellular level. While this is not surprising given the real hazards of carrying out similar research in the living human, confirmation (at least in part) of some of these models must be achieved through human research. The sensitivity of the human organism to a large variety of exogenous/endogenous stimuli will add significant challenges. It may however also lead to the confirmation or modification of previous animal based models, or the construction of competing models of epilepsy. The following research was undertaken in this spirit.

Chapter 3

RESEARCH QUESTIONS

The mature female undergoes cyclic changes in blood levels of ovarian steroids and gonadotropins which regulate her menstrual cycle. Fluctuating blood levels of estrogen, and their interaction with existing secretory/inhibitory feedback mechanisms, appear to be the prime determinants of gonadotropin release. Thus estrogen, originally produced through the aromatization of androgens by F.S.H. (in the ovarian granulosa cells), not only determines oocyte selection and maturation, but also stimulates production of L.H. and progesterone. Both of these hormones are intimately involved in follicular rapture and ovulation.

The rhythmic release of the gonadotropins L.H. and F.S.H. is contingent on the production of Gonadotropin-Hormone-Releasing-Hormone (G.H.R.H.). G.H.R.H. release into the system is in turn influenced by the action of fluctuating blood levels of estrogen and progesterone at the hypothalamo-hypophyseal axis level (as noted above). While the exact sites of action for estrogen and progesterone remain conjectural at best, axonal arborizations of neurons containing G.H.R.H. have been identified in the arcuate nucleus, medial basal hypothalamus, median emminence, posterior pituitary and the limbic system (Silvermann et al., 1977). Thus extensive distribution of relay cells which undergoes cyclic changes in ionic milieu as well as significant changes in activity levels has been demonstrated.

The Schwartzkroin and Wyler (1980) model of epilepsy proposed that every neuron possessed a proclivity to burst firing, although a triggering mechanism or influence may be necessary for initiation. An

unbalanced ionic milieu, they added, could become a triggering mechanism. Prince et al. (1983) proposed that events that caused "... intense synchronization (of neuronal populations), prolonged depolarizations and loss of intrinsic control mechanisms ...", could facilitate ictal discharges. Prince et al. (1984) further added that, both in the hippocampus as well as in the neocortex, "pacemaker cell" populations may spread synchronized burst firing in adjoining connected neuronal populations in what they termed a "cascading effect".

Increased hippocampal activity for the production of G.H.R.H., the presence of increasing levels of estrogen in the ionic milieu and the possible action of estrogen on ionized calcium levels could then synergistically alter the chemical microenvironment. Neuronal arborizations and probable disinhibition (as a result of altered microenvironment) would allow for the recruitment of surrounding neurons through extensive pathways, resulting in ictal phenomena.

The use as subjects, of epileptics refactory to anticonvulsant control, identifies these subjects as particularly at risk of ictal episodes. Their chronic ingestion of anticonvulsants not only alters their calcium metabolism, but also inhibits its entry along the neuron's electro-chemical gradient. This latter function (calcium channel blocking) is the major inhibitory action of some anticonvulsants. While at first hand this may appear beneficial, it could - given a disturbed milieu as well as enhanced hippocampal/hypothalamic excitation - provoke intra-neuronal release of ionized calcium (Rahamimoff et al., 1975; Wong and Prince, 1978). Glial-based (N^+ - K^+)-ATPase clearance of extracellular potassium may be disturbed as a result of previous burst firing activity, or as a result of altered ionic milieu. Thus optimal

conditions for synchronized burst firing would exist (Grisar, 1984; Stahl, 1984).

It is therefore appropriate to seek a possible relationship between levels of estrogen, progesterone and ionized calcium with seizures, during the menstrual cycle of female epileptics. This relationship may be elicited by answering a number of research questions:

(1) Do seizures tend to cluster around specific stages of the menstrual cycle, or are they randomly distributed?

(2) Are the plasma levels of estrogen, progesterone and ionized calcium different when seizure clustering occurs, than levels when no clustering occurs?

(3) Is there a relationship between levels of estrogen, progesterone, ionized calcium and seizures, such that when estrogen levels are elevated, ionized calcium levels are lowered and more seizures occur?

Chapter 4

METHOD

4.1 Introduction

This study addressed three major questions. First, it sought to investigate the possibility that seizures do not occur randomly in the female, but rather in association with the physiology of the menstrual cycle. Second, it sought to establish plasma levels of estrogen, progesterone and ionized calcium both when seizure clustering occurs (if it does), and at other times when no seizures are exhibited during the same cycle. Third, it attempted to correlate plasma levels of estrogen, progesterone and ionized calcium with seizures. The hypothesis proposes that when estrogen levels are elevated, plasma levels of ionized calcium are lowered and more seizures are apt to occur under these circumstances than at other times. The anticonvulsant action of progesterone is seen as mitigating the action of estrogen when this hormone (progesterone) is elevated around the luteal phase of the cycle. Data gathered in an attempt to answer these questions were obtained from a population of mentally retarded female epileptics resident of two mental retardation institutions in southwestern Ontario.

4.2 The Sample

Both groups of prospective subjects had to satisfy a number of criteria prior to becoming eligible for participation in the study.

4.2.1 Eligibility Criteria

Each prospective subject considered for inclusion in the prospective subject frame had to exhibit the following properties:

(1) Be a female.

(2) Be between the ages of 18 and 40 years at the time of

selection.

(3) Had experienced seizures for a minimum of three months prior to selection.

(4) Had been menstruating during the preceding three menstrual cycles prior to selection.

(5) Be without overt osteoclastic/osteoblastic disease prior to selection.

(6) Be free from musculo-skeletal deformity resulting in complete immobility, or rendering antecubital venepuncture difficult.

4.2.2 Definitions

4.2.2.1. Epilepsy

An epileptic seizure is defined for the purposes of this study as "A paroxysmal alteration of intellectual, sensory, motor, autonomic or affective activity, which is time limited (usually under one hour), and presumably associated with neuronal hypersynchronous overactivity." (Alter et al., 1972). Criterion (3), in Section 4.2.1, would eliminate the inclusion of subjects who exhibited epilepsy in association with a sudden acute disorder of presumably short duration. In addition, although the preceding Alter et al. (1972) definition places a time (duration) constraint on a seizure, this in no way eliminates the possibility of regarding a status epilepticus as a series of seizures.

4.2.2.2 Menstrual Cycle

A menstrual cycle is here defined as the elapsed time between the day after onset of menses from the previous cycle to the day of appearance of menstrual flow for the cycle under examination

(Vollman, 1977; Ross et al., 1970). Intra-subject variability in cycle length had been demonstrated by Knaus (1950), Vollman (1956b; 1977), Matsumoto et al. (1962a), Treloar et al. (1967). The closer each subject is to either menarche or menopause, the higher the variability of cycle length. 21 to 35 days is considered normal (Benson, 1980). Subjects who are amenorrheic, as a result of either surgical or chemotherapeutic intervention, were excluded. In addition, subjects with highly irregular menstrual cycles were also excluded, not only to facilitate inter-subject comparison, but also to render blood withdrawal uniform and less discomforting.

4.2.2.3 Osteoblastic/Osteoclastic Disorders

Osteoblastic disorders describe a series of conditions resulting in increased skeletal bone mass. They may result from one of two causes: increased matrix formation typically found in acromegaly, renal failure and osteofluorosis, or decreased calcium resorption as found in hypoparathyroidism and osteoporosis (Golden, 1982).

Osteoclastic disease on the other hand describes a series of conditions in which a loss or reduction of skeletal bone mass is exhibited. Possible etiologic factors are failure of normal bone matrix to mineralise, accelerated calcium resorption and dysfunctional osteoid synthesis (Golden, 1982).

Section 2.6 of this study dealt with factors affecting levels of ionized calcium. Several researchers reported the antagonistic (to calcium resorption) effect of the anticonvulsants phenobarbitone, primidone, and phenytoin sodium (Richens and Rowe, 1970; Dent et al., 1970; Bowden, 1974; Christiansen et al., 1974; Mosekilde et al., 1979; Bell et al., 1979; Aladjem et al., 1980). However this osteoclastic

syndrome is secondary to anticonvulsant use. Exclusion of the subjects exhibiting this syndrome may have effectively denied entry to all subjects currently taking the above anticonvulsants. Thus only prospective subjects demonstrating primary osteoclastic disease were excluded.

Prospective subjects diagnosed as having Cushing's syndrome, hyperthyroidism, ovarian dysgenesis, primary hyperparathyroidism or bone tumors were rejected. In addition, subjects undergoing corticosteroid therapy were also ineligible to participate. Osteoclastic disease may be caused by other factors - e.g. renal failure, severe malnutrition and carcinomatosis - however the subjects' symptomatology in these cases would be sufficient for exclusion from the study. Thus identification of such causes is not deemed necessary, although one subject was excluded shortly after starting data collection following a diagnosis of breast cancer.

4.2.2.4 Non-Institutional Subject Recruitment

Clinical neurologists at the three teaching hospitals in London, Ontario were approached and asked to co-operate in subject recruitment. An examination of records by hospital staff produced 250 female epileptics living in and around London, Ontario who were also between the ages of 18 and 40 years. Further exclusions were made on the basis of:

(a) Residence (subject no longer domiciled in the London area, records unchanged).

(b) Seizure activity (subject's seizures under control).

(c) Gynaecological status (no longer menstruating, highly irregular cycles, birth control use).

(d) Medico-neurological status (had undergone brain surgery,

or been diagnosed with further complicating disorders).

(e) Re-evaluated diagnoses - subjects no longer considered epileptic.

This effectively reduced the prospective subjects' frame to a total of 58 subjects. Each subject was contacted by a mail package which included:

(1) A letter of introduction from the prospective subject's neurologist (Appendix A).

(2) An explanatory letter from the principal investigator (Appendix A).

(3) A consent form (Appendix A).

(4) A return stamped self-addressed envelope.

A total of 5 subjects had agreed to participate, and 10 refused by the expiration of 4 weeks. A reminder (Appendix A) sent to non-respondents at this time was returned (with a negative reply) by a further 5 subjects.

In an attempt to increase participation, the area of recruitment was widened to include most of southwestern Ontario. Female epileptics attending neurology clinics at University Hospital, London, Ontario were personally interviewed - after consent to interview was obtained by their consulting neurologist. A total of 45 prospective subjects agreed to be interviewed over a period of six months, of whom 29 qualified for inclusion. Following explanation of a modified protocol (each subject, on consent, was primarily asked to maintain a menstrual and seizure history for three menstrual cycles to be followed by blood collection), each consenting subject was issued with a kit containing:

(1) A consent form (Appendix B).

(2) A basal body thermometer.

(3) Explanatory written information about the taking and

recording of basal temperature and seizure events.

(4) A number of seizure diaries and temperature recording forms (Appendix B).

(5) A Chemotherapy Recording Form (Appendix B).

The protocol called for daily recording of basal body temperature, and the maintenance of a seizure diary. At the end of each cycle, the appropriately filled forms were to have been returned to the investigator, in supplied self-addressed stamped envelopes. Five of the original 29 consenting subjects carried the task to completion.

In view of the low response and the institution of necessarily stringent quality control measures of data collection, recruitment and use of further non-institutionalised subjects was terminated. Instead, only institutionalised female epileptics were approached and ultimately used.

4.2.2.5 Institutional Subject Recruitment

A survey of all Ontario-based institutions for the mentally retarded yielded four institutions with sufficient numbers of eligible subjects. Each institution was approached with a copy of the study hypothesis and protocol for examination by their respective research committees. Only two institutions (The Oxford Regional Centre and The Southwestern Regional Centre) acceded to further co-operation (Appendix C).

The records of female epileptics at each institution were first examined on the criteria of age, seizure status and drug intake (see Tables 2 and 3). Further exclusions were made on the basis of menstrual history and musculo-skeletal (plegia/paresis) status. Final prospective subjects' pool was reduced to 53 (S.W.R.C. 35; O.R.C. 18). The

Female Epileptics Resident of two Mental Retardation Institutions

Number seizuring/not seizuring by age and hormonal ingestion

S.W.R.C.*

AGE	< 18		18-40		> 40		TOTALS
Birth Control	Yes	No	Yes	No	Yes	No	
SEIZURING	0	4	13	61	0	11	89
NOT SEIZURING	0	0	3	18	0	6	27
							116

* South Western Regional Centre.

Table 2

O.R.C. *

AGE	< 18		18-40		> 40		TOTALS
Birth Control	Yes	No	Yes	No	Yes	No	
SEIZURING	0	2	11	27	2	54	96
NOT SEIZURING	0	0	2	2	0	12	14
							110

*Oxford Regional Centre.

Table 3

identified next-of-kin of each prospective subject were contacted by mail with a kit which included:

(1) A Consent Form (Appendix C).

(2) A letter of introduction from the facility (Appendix C).

(3) A letter of introduction from the investigator (Appendix C).

(4) A stamped self-addressed return envelope.

Seventeen forms permitting research were returned by the next-of-kin of prospective subjects at S.W.R.C. and six by the next-of-kin of subjects at O.R.C., for a total of 23 subjects.

Consent forms returned to the principal investigator from next-of-kin of subjects at the O.R.C. were placed in a locked filing cabinet, with access limited to the principal investigator. Consent forms for residents of the S.W.R.C. were returned directly to the institution and now form part of the resident's permanent record.

4.2.2.6 The Study Population

Although as noted in section 4.2.2.5, 23 next-of-kin permissions were received, the subject's permission to carry out the research superceded all other consents. Each subject was approached individually, and where possible the protocol was explained to her in simple language, with particular emphasis on the blood collection requirements and her right of refusal. There were no refusals or overt resistance from the subjects at O.R.C. Five subjects at S.W.R.C. declined to participate either verbally or by resistance to blood withdrawal. These subjects were immediately withdrawn from further participation. In addition, one subject who had originally agreed to participate was withdrawn after initiation of blood collection, following a diagnosis of cancer.

4.3 Field Work

Receipt of signed consent forms set in motion a re-examination of subject in-patient records. Seizure and menstrual histories - for six retrospective months where possible - were abstracted from each record. Since data were collected from two facilities, a substantial number of miles apart and organizationally different, the co-operation of staff to maintain strict quality assurance in data collection assumed great importance.

4.3.1 Staff Preparation

Each subject's counsellor, as well as any other interested staff member working in the subject's residence, were personally met. The hypothesis, protocol and their involvement in the project were explained. The need for conscientious observation and reporting of seizures, menstrual flow and drug ingestion were emphasized. A previously established centralized system of reporting was explained, and each residence was given a set of written instructions (Appendix D) for further reference.

Actual blood withdrawal was carried out by resident laboratory technicians retained for the purpose. Their participation may have helped reduce apprehension in the subjects, and their expertise must have reduced discomfort considerably.

Laboratory personnel were also met personally. Emphasis was placed on interpreting resistance to blood withdrawal as refusal to participate. A printed set of instructions was left for future reference (Appendix D).

4.3.2 Blood Collection

Individually packaged pre-labelled collection kits were

issued to laboratory personnel engaged in blood collection. Each kit contained:

(1) Sterile 3 and 5 ccs. plastic disposable syringes with screw-top caps.

(2) Two 21G luer needles.

(3) 1 pre-labelled 5 ccs. Royal Blue top "Vacutainer".
1 pre-labelled 7 ccs. Red top "Vacutainer".

(4) Labelled freezer storage bags.

Blood collection commenced the morning following start of menstrual flow for the preceding cycle, and continued on an alternate day basis up to and including the first day of menstrual flow for the cycle under investigation. Blood was withdrawn from the antecubital fossa, using each arm alternately.

Markowitz et al. (1981) demonstrated a circadian calcium rhythm with highest determinations registered daily around 10.00 hours. Thus blood was withdrawn between 08.00 and 10.00 hours with each subject in a fasting state. This effectively removed any food/calcium level interaction and minimized the possibility of confounding time-of-withdrawal effect. However, no attempt was made to restrict prescribed chemotherapy use.

Blood destined for Ca^{++} assay was withdrawn into a Royal Blue top siliconized "Vacutainer" under strict anaerobic technique. Blood for all other assays was withdrawn into a Red top "Vacutainer" with no additives. Blood was taken to the respective facility's clinical laboratory for further preparation after collection.

4.3.3 Sample Preparation and Storage

Whole blood samples were left at ambient temperature for 30

minutes after collection, and then spun in a centrifuge at 2,000 rpms. Serum was aspirated into a plastic syringe via a 21G luer needle (without removal of "Vacutainer" top). The syringe was capped, labelled and frozen. Frozen serum aliquots were transported in a frozen state under refrigeration weekly and stored at -80°C, at the clinical biochemistry laboratories at St. Joseph's Hospital in London, Ontario.

4.3.4 Serum Analysis

All sera were assayed at St. Joseph's Hospital, London, Ontario by technicians retained for the purpose. Commonly used standard laboratory techniques were employed (see Table 4). Aliquots were assayed in duplicate in random fashion to minimize systematic error. 17_{B}Estradiol was measured by radioimmunoassay (RIA) procedures described by Challis et al. (1980). The inter-assay coefficient of variation was 6.0%. Progesterone was also measured by RIA using procedures described by Garfield et al. (1979). The inter-assay coefficient of variation for this assay was 8.0%. All samples (assayed for these hormones) for any one individual were run together.

4.3.5 Potential Sources of Bias

This study, under its present format, contains a number of potential sources of bias. First, it excludes non-institutional female epileptics. These individuals as already discussed were not excluded by choice, but rather by force of circumstance. Whether their serum level of electrolytes, enzymes and hormones are significantly different from those of the study population will remain unknown.

Second, this study made no attempt to recruit non-epileptic females. The cyclically fluctuating serum levels of the variables under investigation may or may not be different in the two populations.

Analytes and Assays carried out on collected Serum with interassay Coefficient of Variation.

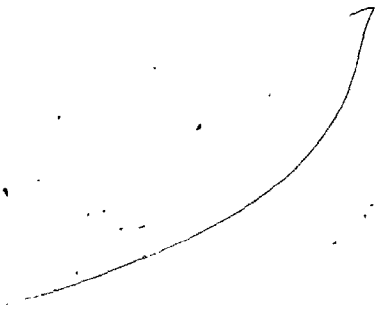
ANALYTE	ASSAY	Coef. of Var.
Ionized Calcium	Flowthrough ion-specific electrode (ORION SS - 20)	1.4 %
Total Calcium	Ortho-cresolphthalein-complexone (Beckman Astra - CPC)	2.6 %
Albumin	Bromcresol Green (Beckman Astra)	3.4 %
Total Protein	Biuret Reaction (Beckman Astra)	2.1 %
Inorganic Phosphate	Ammonium molybdate (Phosphomolybdate)	5.0 %
Alkaline Phosphatase	P-nitrophenyl phosphate (B.M.C. Kit)	6.3 %
Magnesium	Atomic absorbtion spectrophotometry	3.8 %

Table 4

However, inferences may be drawn by comparing results from this study to standard diagnostic ranges and previously reported research. Data collection procedures in this study closely approximated those utilized by Pitkin et al. (1978) in their study of the effects of estrogen and progesterone on serum ionized calcium levels in the normal female.

Third, as discussed previously, a substantial rate of refusal was encountered. Increasingly stringent regulations protective of the individual's privacy made assessment of possible bias introduced by their exclusion impossible to ascertain.

Finally, the small sample size of this study population may have substantially increased the chance of Type II error rate in statistical analysis. Potentially significant results may have been masked by the sample size.



Chapter 5

RESULTS AND DISCUSSION

5.1 Introduction

Results and their discussion will be presented in the same hierarchical order as the research questions posited in Chapter 3. An examination of the subjects' menstrual cycle will precede these discussions.

5.2 The Subjects' Menstrual Cycle

The term "study cycle" denotes the menstrual cycle during which blood samples for electrolyte, hormonal and mineral assay were withdrawn. The length of this study cycle ranged between 22 and 40 days, with a median of 28 days. A number of menstrual cycles preceding the study cycle were examined from the subjects' in-patient records. Their length was compared to that of the study cycle. On average, 4 cycles per subject were examined, with subjects in the higher intellectual functioning levels (who did not require routine personal hygiene monitoring) contributing rather less. With the exception of subject number three, no subject demonstrated a statistically significant difference in length of cycle between the study cycle and the preceding cycles (see Table 5).

5.2.1 Menstrual Cycle Stages

Physiologically, ovulation has been associated with the occurence of an L.H. peak, attendant on a sustained (longer than 36 hours in duration) rise in estrogen levels above 200 pg/ml. (Young and Jaffe, 1976; Eddy and Pauenstein, 1979; Fritz and Speroff, 1982; El Sheikh et al., 1984).

Chronologically, the L.H. peak occurs from 36 to 50 hours after

Comparison of Cycle length in days between Pre-study Menstrual cycles and Study Menstrual Cycle.
Differences in length tested at the 0.05 level of Significance.

Subjt. #	Pre-Study Cycle Length in Days	Study-Cycle Length in Days	Signif.
1	31,33,32,35,29	36	P>0.05
2	38,35,37,35,40	36	P>0.05
3	17,19,17,22	40	P<0.01
4	27,26,25,33,31	34	P>0.05
5	34,32	34	P>0.05
6	34,31,33,30	34	P>0.05
7	21,25	22	P>0.05
8	28,31,31,29,32, 29	28	P>0.05
9	30,32,29,31	34	P>0.05
10	32,25,29,28,37	28	P>0.05
11	20,26,25,24,23	24	P>0.05
12	22,35,21,26	28	P>0.05
13	31,27,26	28	P>0.05
14	30,24,21,29,34	28	P>0.05
15	33,30,29,32	34	P>0.05
16	25,22,22,23,26	30	P>0.05

Table 5

the critical rise in estrogen levels described above. Ovulation, if it is to occur, takes place some 12 hours after the L.H. peak, or some 24 to 36 hours following the estrogenic peak (Pauerstein et al., 1978; Eddy and Pauerstein, 1979; Garcia et al., 1981; Fritz and Speroff, 1982).

Luteinizing hormone levels were not assayed in this study, since determination of ovulation was deemed unimportant. For the purpose of this study, determinations for each subject were forced into a thirty day cycle, with the estrogen peak assigned to day "0". Thus the cycle was subdivided into three stages. Day 0 +/- two determinations was labelled the Ovulatory stage, determinations -3 to -7 labelled the Follicular stage, and determinations +3 to +7 the Luteal stage. Since determinations were undertaken on alternate days, the resultant length of this artificial model is 30 days.

Seizures were registered on a daily basis. Thus some seizures occurred on days when no blood withdrawals were undertaken. These events were lagged forward one day to conform with the model.

5.3 Seizures During the Study Cycle

Difficulties with subject recruitment mandated the inclusion of subjects with different types of epilepsy. Thus two subjects experienced both Absence as well as Grand Mal seizures. Absence attacks on any particular day, regardless of number (unless they constituted a status epilepticus), were recorded as one seizure event. Two subjects who experienced a Grand Mal status epilepticus during the study cycle were arbitrarily assigned 10 seizure events each for that occurrence. This number may be either under- or over-representative of the real number of seizures exhibited, but is seen as the best possible

representation of an event, which is particularly difficult to quantify in field conditions.

Seizures experienced by each subject in each of the three study cycle stages are presented in Table 6 and Figure 9. The Poisson-like distribution of the seizures necessitated a square root transformation of the data (seizures) in their analyses.

Although there are obvious differences in the numbers of seizures occurring in each of the cycle stages, tests of statistical significance of these differences must take into account the "repeated measurement" quality of the data. Donner (1984, 1985) stated that repeated measurements induce clustering of determinations on the dependent variable. Standard statistical techniques of hypothesis testing do not distinguish between the variances within and between these clusters. This leads to possible underestimation of the true variance, and to spuriously significant statistical results.

A correction factor (C) capable of adapting the generally used Goodness of Fit chi-square statistic was suggested by Brier (1980). An alternative correction factor estimator using the Snedecor and Cochran (1980) ANOVA technique was developed by Donald (1984), who found in a simulation study that the latter correction factor was less biased and had a lower variance than that of Brier, especially when used to test small samples (Appendix E).

The goodness of fit chi-square used to test the difference in seizures for each cycle stage was thus corrected by a factor $\hat{C}_A$ developed by Donald.

The total number of seizures for all subjects occurring in the follicular stage of the cycle was significantly different from those

Number of Seizures Exhibited during the Study Cycle

Raw numbers by Subject, Cycle Day, Cycle Stage, with totals by Cycle stage.

	Cycle-Stage														
	Follicular					Ovulatory					Luteal				
Day	-7	-6	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	+6	+7
Sbjt.#															
1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0
3	1	2	1	0	0	0	0	0	0	0	0	0	0	10	0
4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	1	0	0	0	0	0	0	2	0	0	1	1	0	0
6	0	2	0	0	1	0	0	0	0	0	0	0	1	1	2
7	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
8	0	0	0	0	0	0	0	0	1	1	2	1	1	0	0
9	0	3	0	2	1	1	0	0	0	0	0	0	0	0	0
10	2	1	2	10	10	0	1	0	1	5	1	2	1	2	0
11	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
12	0	0	0	1	1	0	0	0	0	1	0	0	1	4	0
13	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0
14	2	2	3	0	0	0	0	0	1	1	2	0	1	4	0
15	0	0	0	1	2	0	0	0	0	0	2	0	0	0	0
16	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
Totals	54					20					46				

** Subject #2 excluded at the analyses stage due to an aberrant Estrogen distribution incapable of fitting the model.

Table 6

Distribution of Seizures by Cycle Day

Total number of seizures for all subjects by cycle day forced into a 30 day cycle.

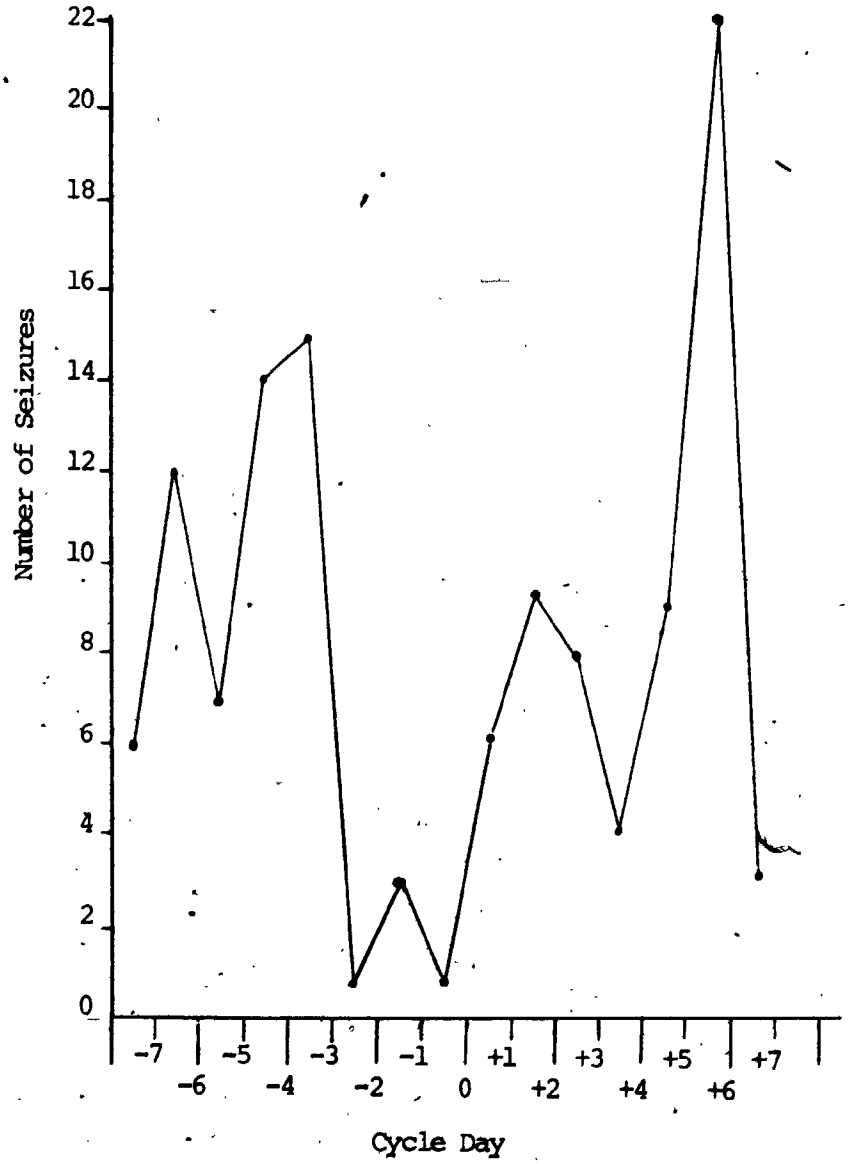


Figure 9

occurring in the ovulatory stage - adjusted χ^2 with 1 d.f. was 10.07, $P < 0.01$. Seizures occurring in the luteal stage were significantly more than those occurring in the ovulatory stage - adjusted χ^2 with 1 d.f. was 7.276, $P < 0.01$. Not unexpectedly then, seizures occurring in both the follicular and Luteal stages combined were significantly more than those occurring in the Ovulatory stage alone - adjusted χ^2 with 1 d.f. was 15.00, $P < 0.001$. This is strong evidence that seizures were not randomly distributed through the cycle. Rather, 43.5% of total seizures were exhibited during the follicular stage, 40% during the luteal stage, and only 16.5% occurred during the ovulatory stage. Eight subjects (53%) exhibited seizures during their menstrual flow, but none of these subjects demonstrated catamenial epilepsy exclusively (catamenial epilepsy is defined as epilepsy exhibited exclusively during the menstrual flow stage of the cycle).

5.4 Hormones During the Menstrual Cycle

Serum estradiol (17_B) for the whole of the cycle in all subjects ranged from a low of 13pg/ml to a high of 573pg/ml, with a mean of 136.1pg/ml and S.E. = 6.82pg/ml. Mean values for estradiol 17_B for each of the cycle stages previously described were: follicular stage 67.976pg/ml, S.E. = 4.987; ovulatory stage 202.0pg/ml, S.E. = 12.540; luteal stage 142.536pg/ml, S.E. = 11.279. These values were within the standard ranges expected for each cycle stage (Krupp et al., 1985); thus levels of estrogen fell within accepted normal values.

Serum progesterone in all subjects for the whole cycle ranged from a low of 166 ng/dL to a high of 1196 ng/dL with an S.E. of 15.4516 ng/dL. Mean values of progesterone for each of the cycle stages were: follicular stage, 67.58 ng/dL, S.E. = 11.32; ovulatory stage,

112.86 ng/dL, S.E. = 16.9; and luteal stage, 343.20 ng/dL, S.E. = 37.79. These determinations like those for estradiol also fell within the standard ranges expected, although mean progesterone for all subjects during the luteal stage approached the lower scale of the range (Krupp et al., 1985).

Serum was not assayed for levels of L.H. and F.S.H. Cycles were instead centred around their estrogen peak, and data analysis adapted to account for higher intra-subject correlations resultant from repeated measurements on each subject. Data were analyzed using ONEWAY analysis of variance techniques described by Kim and Kohout (1975), and adapted by using the correction factors described by Donner (1984).

5.4.1 Effect of Estrogen on Ionized Calcium

Previously cited research dealing with the effects of levels of estrogen on levels of ionized calcium during the menstrual cycle was carried out on subjects with equal length cycles, and thus coincident estrogen peaks (Pitkin et al., 1978). Five subjects in the present study exhibited 28 day cycles and coincident estrogen peaks. The relationship of ionized calcium levels to estrogen levels during the cycle of these five subjects is presented in Figure 10. The slope b_1 was -1.4734×10^{-4}. The unadjusted and adjusted standard errors of this slope were recorded at 1.07×10^{-4} and 1.03×10^{-4} respectively. A two-tailed t with 67 d.f. was computed at 1.426, $P > 0.10$ (Table 7).

When this relationship was examined in all the subjects combined, the slope b_1 emerged at 2.66×10^{-4} and the unadjusted/adjusted standard errors of b_1 computed at 6.0×10^{-5} and 7.0×10^{-5} respectively. A two-tailed t with 227 d.f. was recorded at 3.881, $P < 0.001$, Pearson's Rho = 0.27, $P < 0.001$ (Table 7).

The Relationship of Levels of Estrogen to Levels of Ionized Calcium in 5 females with 28 day cycles.

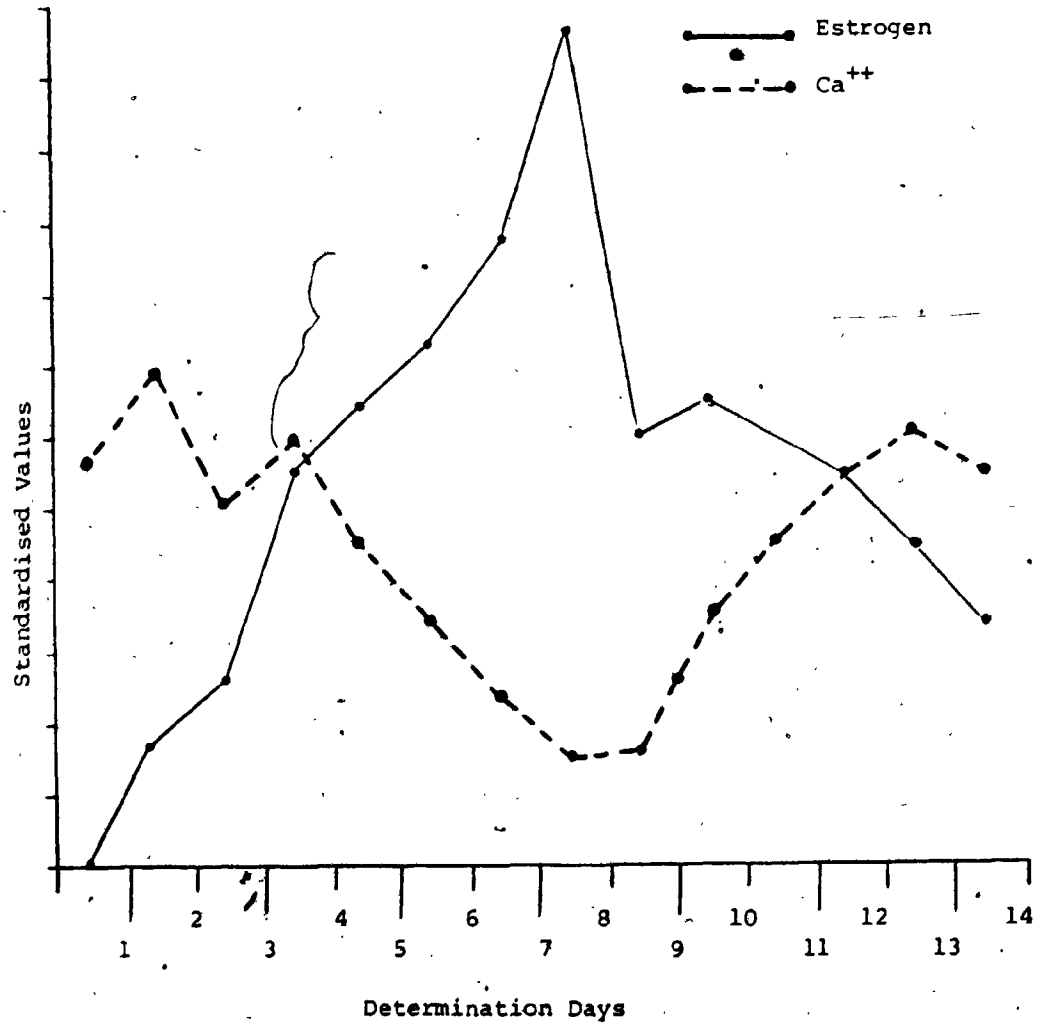


Figure 10

Results of Regression Analysis with Correction Factor for Repeated Measurements (Donner, 1984): Estrogen with Ionized Calcium.

Number of Subjects = 5
Mean Cluster Size (Unadjusted) = 13.800
Mean Cluster Size (Adjusted) = 13.650
F Ratio = 3.52
Rho Sub X = -0.031 Rho-Hat = 0.156
Correction Factor = 0.969

$B_1 = -1.474 \times 10^{-4}$

Standard Error : Unadjusted Error = 1.07×10^{-4}
Adjusted Error = 1.03×10^{-4}

t statistic = 1.426 $p > .10$
(67 d.f.)

Number of Subjects = 15
Mean Cluster Size (Unadjusted) = 15.267
Mean Cluster Size (Adjusted) = 15.051
F Ratio = 4.71
Rho Sub X = 0.141 Rho-Hat = 0.193
Correction Factor = 1.18

$B_1 = 2.66 \times 10^{-4}$

Standard Error : Unadjusted Error = 6.0×10^{-5}
Adjusted Error = 7.0×10^{-5}

t statistic = 3.881 $p < .001$
(227 d.f.)

Table 7

Thus the t statistic for the five subjects, although not significant, can not be regarded as spurious. Rather it appears to lose significance directly as a result of a small sample size. A larger n (i.e. in all the subjects combined) produced a highly significant statistical relationship. It can therefore be suggested that estrogen exercises a significant effect on levels of ionized calcium in all the subjects combined, in spite of variable cycle lengths which tend (when grouped together) to increase intra-subject correlation or reduce intra-subject variability. A t statistic, already approaching significance in the five subjects, may become highly significant with the addition of more subjects with the same cycle lengths.

5.4.2 Effects of Estrogen on Seizures

A similar relationship (to that described in section 5.4.1) is demonstrable when the effects of estrogen on seizures is sought. In the five subjects, the slope b_1 was computed at -1.044×10^{-3} with an unadjusted/adjusted standard error of the slope computed at 6.9×10^{-4} and 6.4×10^{-4} respectively. A one-tailed t with 67 d.f. registered at 1.621, $P = 0.06$ (Table 8).

When all the subjects are examined together, the slope b_1 was computed at -3.197×10^{-3} with an unadjusted and adjusted standard error of 2.7×10^{-4} and 6.1×10^{-4} respectively; t with 227 d.f. = 5.241, $P < 0.001$, Pearson's Rho = -0.23, $P < 0.001$ (Table 8).

Once again the relationship between levels of estrogen and seizures in the five subjects is marginally below significance level. The strength of the significance of t in all the subjects combined suggests a strong negative relationship between levels of estrogen and seizures. This relationship is further reinforced by the previously

Results of Regression Analysis with Correction Factor for Repeated Measurements (Donner, 1984): Estrogen with Seizures.

Number of Subjects = 5
Mean Cluster Size (Unadjusted) = 13.800
Mean Cluster Size (Adjusted) = 13.650
F Ratio = 8.0
Rho Sub X = -0.031 Rho-Hat = 0.339
Correction Factor = 0.930

$B_1 = -1.044 \times 10^{-3}$

Standard Error : Unadjusted Error = 6.9×10^{-4}
Adjusted Error = 6.4×10^{-4}
t statistic = 1.621 $p > .10$
(67 d.f.)

Number of Subjects = 15
Mean Cluster Size (Unadjusted) = 15.267
Mean Cluster Size (Adjusted) = 15.051
F Ratio = 5.19
Rho Sub X = 0.780 Rho-Hat = 0.378
Correction Factor = 1.196

$B_1 = -3.197 \times 10^{-3}$

Standard Error : Unadjusted Error = 2.7×10^{-4}
Adjusted Error = 6.1×10^{-4}
t statistic = 5.241 $p < .05$
(227 d.f.)

Table 8

discussed seizure distribution in section 5.3 and Table 6. It is also felt that once again an increase in sample size of subjects with equal length cycles would result in a much stronger statistical relationship.

5.4.3 Effects of Ionized Calcium on Seizures

Ionized calcium in all subjects combined exhibited a range between 0.858 mmol/L and 1.422 mmol/L, with S.E. = 0.006 mmol/L. The ranges recorded by these subjects lie marginally outside the expected standard values at both ends of the spectrum: Lower Range = Expected 1.05 mmol/L, Observed 0.858 mmol/L; Upper Range = Expected 1.3 mmol/L, Observed 1.422 mmol/L (Krupp et al., 1985). These differences are not however statistically significant; χ^2 with 1 d.f. = 0.85, $P > 0.10$.

Previous analyses reported in section 5.3 demonstrated that fewer seizures occurred during the ovulatory stage of the cycle. In addition, ionized calcium is at a nadir during the ovulatory stage of the cycle, consequent to a rise in levels of estrogen.

The relationship between seizures and ionized calcium in the five subjects previously identified is presented in Table 9 and Figure 11. The slope b_1 = 0.84532 with an unadjusted/adjusted standard error of b_1 = 0.787524 and 0.914025 respectively; t with 67 d.f. = 0.925, $P > 0.10$.

This relationship was not improved by the inclusion of more subjects as demonstrated in other previously presented relationships. In all subjects combined, b_1 = 0.570, and the unadjusted/adjusted standard errors of the slope were 0.381 and 0.589 respectively, Pearson's Rho = 0.09, P = 0.16. Statistical significance is not approached in either the five subjects or all the subjects combined. This relationship is reinforced by previously reported results in this and preceding sections.

Results of Regression Analysis with Correction Factor for Repeated Measurements (Donner, 1984): Ionized Calcium with Seizures.

Number of Subjects = 5
Mean Cluster Size (Unadjusted) = 13.800
Mean Cluster Size (Adjusted) = 13.298
F Ratio = 8.41
Rho Sub X = 0.079 Rho-Hat = 0.358
Correction Factor = 1.161

$B_1 = 8.45 \times 10^{-1}$

Standard Error : Unadjusted Error = 7.87×10^{-1}
Adjusted Error = 9.14×10^{-1}

t statistic = 0.925 $p > .30$
(67 d.f.)

Number of Subjects = 15
Mean Cluster Size (Unadjusted) = 15.267
Mean Cluster Size (Adjusted) = 14.723
F Ratio = 5.12
Rho Sub X = 0.464 Rho-Hat = 0.882
Correction Factor = 1.546

$B_1 = 5.70 \times 10^{-1}$

Standard Error : Unadjusted Error = 3.81×10^{-1}
Adjusted Error = 5.99×10^{-1}

t statistic = 0.967 $p > .30$
(227 d.f.)

Table 9

The Relationship of Levels of Estrogen and Ionized Calcium to Seizures in 5 females with 28 day cycles.

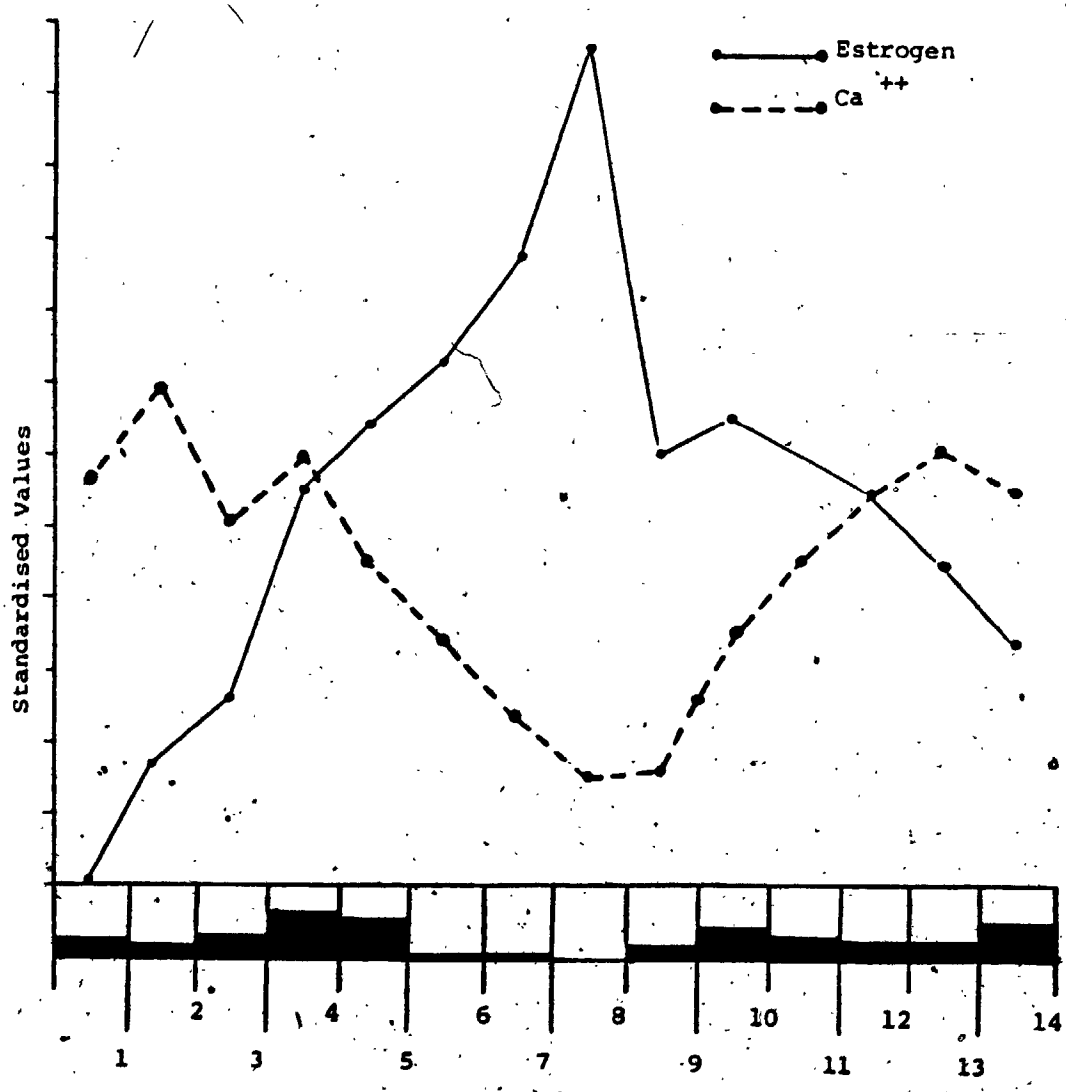


Figure 11

It therefore appears that low levels of ionized calcium encountered during the ovulatory stage of the cycle in these subjects do not induce an increase in seizure activity. The last hypothesis formulated in Chapter 3 of this study can not then be supported; neither can the lack of statistical significance be attributed to the sample size.

5.4.4 Ancillary Results

5.4.4.1 Effect of Estrogen on Progesterone

Progesterone levels are generally stable in the follicular phase of the menstrual cycle - between 20-150 ng/dL - and rise dramatically in the luteal phase to between 300 and 2400 ng/dL (Krupp et al., 1985). Mean progesterone level for all subjects combined in the previously defined cycle stages were recorded at: follicular stage, 67.580 ng/dL; ovulatory stage, 112.860 ng/dL; luteal stage, 343.800 ng/dL. The presence of subjects with anovulatory cycles, and thus lower luteal progesterone levels, decreased the mean progesterone levels in this cycle stage considerably. The effect of estrogen on progesterone in all subjects exhibited a slope b_1 of 3.665 with an unadjusted/adjusted standard error of the slope of 1.484 and 1.730 respectively; t with 227 d.f. = 2.118, $P < 0.05$.

Results for the same relationship examined in the same subjects, but categorized by cycle stage, are reproduced in Table 10. High intersubject variation at the luteal stage coupled with low intrasubject variation produced a $t = 1.751$, $.10 > P > .05$. Progesterone in these subjects is thus behaving as expected, although inclusion of subjects with anovulatory cycles tended to reduce mean levels in the luteal stage.

5.4.4.2 Effects of Progesterone on Seizures

Previously cited research posited an anticonvulsant

Regression Analysis of Estrogen with Progesterone by Cycle Stage with Correction Factor for Repeated Measurements (Donner, 1984)

Estrogen with Progesterone,

	Follicular Stage	Ovulatory Stage	Luteal Stage
Observations	229	229	229
A.M.C.S.	5,284	5,070	4,447
F Ratio	3,15	1,56	7,10
C.Factor	1,196	1,039	1,276
B_1	10,440	-1,743	8,826
Unadj. Std. Err.	2,253	1,541	3,949
Adaj.	2,695	1,601	5.040
T- Statistic (227 d.f.)	3,874	1,088	1.751
p Value	< .05	> .10	> .10

LEGEND : A.M.C.S. = Adjusted Mean Cluster Size
C.Factor = Correction Factor
B_1 = The slope

Table 10

action to progesterone. No conclusive evidence exists about its mode or route of action. The action of progesterone on seizures was then first examined in all subjects combined (Table 11), where no significant relationship was demonstrable. The same relationship examined by cycle stage is presented in Table 12. No statistically significant relationship was found for any of the cycle stages; however the highest level of significance ($t = 0.819$, $P > 0.10$) was registered in the ovulatory stage when fewer seizures (than in either of the other stages) were recorded.

In these subjects, progesterone does not appear to have any significant effect on seizures.

5.4.4.3 Effects of Ionized Calcium on Alkaline Phosphatase

Alkaline phosphatase levels are an index of calcium homeostasis. Thus, when calcium levels are lowered, reactive increases in levels of this enzyme tend to occur. Mean alkaline phosphatase in all subjects combined was recorded at 92.672 U/L, which lies within the expected normal limits of 30-115 U/L (Krupp et al., 1985). This result is not altogether surprising since previously discussed levels of ionized calcium in this population were themselves within normal ranges. The relationship of ionized calcium to this enzyme examined in all subjects combined emerged as expected with a slope b_1 of -52.360, and an unadjusted/adjusted standard error of the slope = 16.948 and 43.624 respectively; t with 227 d.f. = 1.200, $P > 0.10$.

A negative if not statistically significant relationship exists between ionized calcium and this enzyme.

Mean levels of alkaline phosphatase by cycle stage for all subjects combined reflect very little difference: follicular = 94.171 U/L;

Results of Regression Analysis with Correction Factor for Repeated Measurements (Donner, 1984): Progesterone with Seizures.

Number of Subjects = 15

Mean Cluster Size (Unadjusted) = 15.267

Mean Cluster Size (Adjusted) = 15.031

F Ratio = 4.79

Rho Sub X = 0.161 Rho-Hat = 0.201

Correction Factor = 1.207

B_1 = 0.00

Standard Error: Unadjusted Error = 0.000
Adjusted Error = 0.000

t statistic = 0.686 $p > .10$
(227 d.f.)

Table 11

Regression Analysis of Progesterone with Seizures by Cycle Stage with Correction Factor for Repeated Measurements (Donner, 1984).

	Follicular Stage	Ovulatory Stage	Luteal Stage
Observations	229	229	229
A.M.C.S.	5.285	5.111	4.420
F Ratio	6.140	1.480	1.000
C.Factor	1.196	1.039	1.276
B_1	-4.0×10^{-5}	2.8×10^{-5}	1.0×10^{-6}
Std. Err. Unadj.	5.0×10^{-5}	2.0×10^{-5}	2.0×10^{-6}
Std. Err. Adj.	7.0×10^{-5}	2.0×10^{-5}	2.0×10^{-5}
T- Statistic (227 d.f)	0.559	0.819	0.053
p Value	$> .10$	$> .10$	$> .10$

LEGEND : A.M.C.S. = Adjusted Mean Cluster Size
C. Factor = Correction Factor
B_1 = The slope

Table 12

ovulatory = 94.678; luteal = 88.681 U/L. What is, however, instructive are the variances of these means which decrease with time (cycle stage), indicative of a physiologic process reactive to lowered ionized calcium levels observed during the ovulatory stage.

This process is further underlined when the relationship of alkaline phosphatase levels to seizures is examined by cycle stage (Table 13). It appears that the only statistically significant relationship exhibited between seizures and this enzyme occurs during the luteal stage of the cycle, after the observed ionized calcium trough. This once again points to a reactive process of alkaline phosphatase.

5.4.4.4 Summary of Results

Seizures in these fifteen subjects were not randomly distributed throughout their menstrual cycle. Instead, the majority of seizures occurred during the defined follicular and luteal stages of their cycle. No subject exhibited purely catamenial epilepsy.

The highest levels of estrogen were found at day "0" just prior to presumed ovulation, and to a lesser degree mid-luteally. This conforms to previously established cyclic patterns for this hormone. In addition, mean estrogen levels for each of the defined cycle stages fell within the accepted normal ranges for the population.

A highly significant negative relationship between estrogen and seizures was recorded. This underscores the distribution of seizures during the study cycle. It also suggests that when estrogen levels were at their highest, fewer seizures occurred. However when estrogen levels were climbing (in the follicular stage) and still relatively high mid-luteally (because of the secondary mid-luteal estrogen peak) more seizures occurred. Then the action of estrogen on seizure

Regression Analysis of Alkaline Phosphatase with Seizures by Cycle Stage with Correction Factor for Repeated Measurements (Donner, 1984).

	Follicular Stage	Ovulatory Stage	Luteal Stage
Observations	229	229	229
A.M.C.S.	5.113	4.866	4.300
F Ratio	5.170	1.330	0.880
C. Factor	1.622	1.103	0.958
B_1	5.12×10^{-3}	1.66×10^{-3}	4.27×10^{-3}
Std. Err. Unadj.	2.03×10^{-3}	1.30×10^{-3}	2.27×10^{-3}
Std. Err. Adj.	3.29×10^{-3}	1.44×10^{-3}	2.17×10^{-3}
T- Statistic (227 d.f.)	1.555	1.148	1.962
p Value	> .10	> .10	< .05

LEGEND : A.M.C.S. = Adjusted Mean Cluster Size
C. Factor = Correction Factor
B_1 = The Slope

Table 13

generation is confirmed. In addition, the results suggest that estrogen is not the sole activator of burst firing activity. Some other variable or variables exert a mitigating effect. Ionized calcium appears to be one of these variables.

The effects of estrogen on ionized calcium has already been discussed. However its cyclically fluctuating nature had only been proposed once by Pitkin et al., (1978). These results confirm the observations of Pitkin et al. The importance of this confirmation emerged when the effects of ionized calcium on seizures was examined. Contrary to the proposed hypothesis, there appears to be no relationship between low levels of ionized calcium and seizures. In fact, while ionized calcium levels were at their lowest during the defined ovulatory stage, fewer seizures occurred. Then it seems reasonable to suggest that low levels of ionized calcium exerted a beneficial effect, probably mitigating the action of estrogen. During both the follicular as well as the luteal stages of the study cycle, ionized calcium levels were generally stable. At this time more seizures occurred.

Past research had suggested that progesterone exerted a moderating effect on the actions of estrogen. Levels of progesterone tend to rise rapidly after ovulation to a mid-luteal peak. However, mid-luteal levels of progesterone are directly governed by the presence or absence of ovulation. When a viable oocyte is not produced, levels of progesterone tend to remain relatively stable at pre-ovulatory levels. No specific range in levels of progesterone has ever been promoted as beneficial or deleterious to seizure activity. Results observed from these study subjects suggest that most of the subjects experienced anovulatory cycles, since their mean levels of progesterone mid-

luteally fell towards the lower limit of the normal scale. This fact was further confirmed by a statistically non-significant relationship between progesterone and seizure activity.

Chapter 6

DISCUSSION

6.1 Introduction

Scientific research has several mandates to fulfill. Discovery and perhaps more importantly the search for causal connections and the formulation of paradigms that lead to discovery are two of the most important ones.

This last mandate is nowhere more applicable than in the field of human neurology. Because of its very nature, research in this area is often carried out on animal models, or morbid tissue or by inference through indirect observation. Rapid, sometimes acute but more often subtle changes in homeostasis are often unidentifiable under prevailing conditions. Thus much information available today is crude in nature.

This study sought a causal relationship between ionized calcium, estrogen, progesterone and seizures during the menstrual cycle of female epileptics. It was based on previously reported (Chapter 2) research which in isolation and together provided me with the necessary curiosity to formulate the research questions.

6.2 The Sample Population

The study population was made up of mentally retarded female epileptics resident of two mental retardation institutions in south-western Ontario. The small sample size is representative not only of the difficulty to attract sufficient numbers of consenting subjects (either institutionalized or otherwise), but more importantly of the care taken to respect the prospective subjects' wishes. Thus several precious subjects were rejected when resistance to venepuncture was

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Dear

Mr. John Jacono is a doctoral student in the department of Epidemiology and Biostatistics at the University of Western Ontario. He plans to do research about epilepsy in females and has asked me to help him find women who may be willing to help him.

I am therefore sending you this letter to introduce him. Mr. Jacono has included an explanation of his study for you to read. If after reading it, you decide that you would like to participate in his study, please follow his instructions.

Mr. Jacono is known to me, and will be carrying out his research under the guidance of experts in the field.

Many thanks.

Sincerely,

W.T. Blume, M.D.

WTB:r

exhibited; in spite of parental and institutional consents.

6.3 The Nature of the Sample Population

Questions must no doubt arise about intuitive differences between institutional retarded female epileptics and other "normal" female epileptics. This study dealt with physiologic rather than mental, psychic or performance variables. Thus, whether or not physiologic differences between these two groups exist or not will not be answered here. What is readily apparent from the preceding analyses section is the fact that all physiologic variables observed fell within the normal ranges for all females. This appears to indicate that at least for the variables under study, these subjects were no different than any other females.

6.4 Data Collection

Staff members at each institution normally responsible for the daily care of the subjects were involved in data gathering on a voluntary basis. Of note, none of the subjects' personal counsellors refused to participate. Problems with data gathering usually focus on the possibility of over-reporting or padding of data. Over-reporting of seizures in the study population is patently not a problem, since the least number of seizures were reported in the cycle stage where they would have been most desireable. This does not of course eliminate the possibility of under-reporting. Reporting accuracy must be regarded with at least some suspicion when different data collectors are involved. However the close proximity of the counsellors and their expressed interest to participate in the study may have lessened this considerably.

Blood collection and assay were carried out by retained professionals using standard diagnostic techniques and machinery. Error in this

instance could have been introduced through both human and machine error, but is probably no bigger than that found in normal hospital investigative or diagnostic practice. Its diminution - were it possible - would have been impractical given the limited amount of serum available for assay.

6.5 Seizure Distribution During the Cycle

Seizures were not randomly distributed during the menstrual cycle under examination. More seizures were observed during the defined follicular and luteal stages than during the ovulatory stage. It will be recalled that seizure recording was undertaken on a daily basis, while blood collection was carried out on an alternate day basis. Thus seizures recorded when no blood was collected were moved forward one day. Re-examination of the data with lagging of seizures one day backwards (from their original day of occurrence) was undertaken as a precautionary measure. This shift contributed no significant changes to the results reported.

Attribution of 10 seizure events to two subjects who experienced a "status epilepticus" may also raise some objections. Subject number 10 contributed two such events to the seizure total for the follicular stage of the cycle, while subject number 3 contributed one event to the luteal stage total. It may be argued that these events inflated the two stages of the cycle considerably. Altering the number of seizures assigned to these events from 10 to 1 produced the following results: follicular stage with ovulatory stage = adjusted χ^2 with 1 d.f. of 3.015, $.10 > P > .05$. Luteal stage with ovulatory stage = adjusted χ^2 with 1 d.f. of 3.58, $.10 > P > .05$, given such a dramatic change this is not surprising. In reality a ten seizure

ascription to a status epilepticus may be considered rather low, given the time necessary to identify and react to this manifestation.

6.6 Effects of Estrogen on Ionized Calcium

The antagonistic action of estrogen to bone resorption of calcium had been noted in the past, in conjunction with post-menopausal osteoporosis. However in 1978 its cyclic nature was also described by Pitken et al. An attempt to confirm this relationship undertaken by Baran et al. (1980) failed due to inadequate data collection. Of interest here is the fact that this relationship proved to be statistically non-significant in the five subjects with equal length cycles. This in spite of a strongly suggestive graphic configuration (Figure 9). The same was not true in all the subjects combined where this relationship proved strongly significant. Of note is the fact that Pitkin et al. (1978) would have been unable to use the adjusted statistical techniques used in this study. Thus their results may have been artificially high in terms of levels of significance.

Adjusted statistical techniques were used in the present study and it can be concluded that the small sample size (the five equal length cycle subjects) contributed to a loss of power. Confirmation of the cyclic fluctuation of ionized calcium levels will attain greater importance in the following sections.

6.7 Effects of Ionized Calcium on Seizures

It became evident quite early in the data analyses stage that the majority of seizures took place in the defined follicular and luteal stages of the cycle under investigation. Only a small proportion (16.5%) of the total actually occurred during the ovulatory phase when estrogen levels were at a peak, and ionized calcium levels at their

nadir.

In fact the majority of the seizures appear to occur while estrogen levels are elevated and ionized calcium levels relatively stable, in both the follicular and luteal stages of the cycle. The physiological mechanisms underlying these changes may in fact furnish the answer to the seizure distribution phenomenon. In so doing, construction of a new model of seizure causation during the female menstrual cycle may become possible.

It will be recalled that gonadotropin production levels are controlled by a feedback system operating between the hypothalamo-hypophyseal axis. The hypothalamic region and its neuronal arborizations which reach as far as the arcuate nucleus, medial basal hypothalamus, median emminence and the limbic system, undergo dramatic cyclic changes in activity levels as well as changes to their ionic micro-environment (Silvermann et al., 1977). This is necessary for the release of G.H.R.H. which modulates gonadotropin release.

Localised neuronal estrogenic effects, increased neuronal activity and a change in ionic milieu would combine and facilitate spread of synchronized burst action potentials (Prince et al., 1983, 1984). This paradigm fits and compliments the model advanced by Schwartzkroin and Wyler (1980). Levels of ionized calcium and estrogen are both quantitatively sufficient during both the follicular as well as the luteal stages to support this proposed chain of events. The mitigating action of progesterone is probably governed by a dose-response relationship, such that viable oocyte production would increase mid-luteal progesterone production which may temper the estrogenic effect. On the other hand, an anovulatory cycle would severely reduce mid-luteal

progesterone levels and probably increase propensity to burst firing activity.

Results obtained from this study pop[illegible]lo[illegible]appear to promote such an explanation. In addition, the smalle[illegible]ber [illegible] seizures exhibited by these subjects at the ovulat[illegible]ycle appears to suggest that contrary to other [illegible], previously cited, substantially lowered levels of [illegible]observed during this period (in these subjects) may have [illegible]alutary effect.

6.8 Ancillary Results

Research conducted in the 1960's and 1970's reported conflicting results about the bone mineral status of epileptics. Several reports previously cited found evidence of osteoclastic disease in institutionalized epileptics. Others found no differences between their subjects and non-epileptic control groups. Several theories were advanced by both camps, although it was generally accepted that some anticonvulsants promoted calcium and vitamin D malabsorption syndromes (see Section 2.8).

Subjects in this study were examined for this phenomenon by monitoring of their alkaline phosphatase levels. Alkaline phosphatase levels did rise during the luteal stage of the cycle in reaction to ionized calcium troughing during the ovulatory stage. However, as already discussed in section 5.4.4.3, mean alkaline phosphatase levels for all subjects combined fell towards the middle of the expected normal ranges.

It is uncertain whether this represents an improvement in care (from that accorded to subjects in whom osteoclastic disease was found), or a confirmation of later research which minimized osteoclastic syndromes in these persons. In all probability, both reasons could be partly true.

Chapter 7

IMPLICATIONS FOR THE FUTURE

7.1 Introduction

This study owes its inception to the realization that more females than males experience de novo epilepsy in their peri-pubertal period. Intuitive explanations only served to accentuate this anomaly, while previously reported research pointed to a number of sometimes conflicting relationships. This state of affairs prompted a search for a possible causal relationship. However, the magnitude of the problem of a long term prospective cohort study became apparent very quickly. Instead suggestive evidence was sought in gynaecologically mature females.

7.2 The Results

Results in this study were obtained by the use of statistical techniques of "double precision" magnitude. Thus spurious statistical significance is avoided in spite of the repeated measurement nature of the data and the small sample size. It would then seem appropriate to confirm these results in a larger population. The subjects in this study all suffered from Primary Generalized Epilepsy. The behaviour of other types of epilepsy examined under the same conditions could provide further insights.

The hormonal differences exhibited by ovulatory/anovulatory cycles have been discussed especially in reference to the mid-luteal levels of progesterone. It will be recalled that progesterone is suspected of having anticonvulsant properties. Therefore further research in the possible difference in seizures during ovulatory/anovulatory cycles by cycle stage could provide clarification of the role of progesterone.

Of particular importance would be the determination of a quantitative range for this hormone associated with beneficial effects on seizures. In addition, testosterone is reputed to possess similar properties to progesterone in the male. Thus a comparison between a male and female cohort becomes highly important.

7.3 Relationship of Study to Adolescent Epilepsy

It was stated at the outset that the original intent was to enquire into reasons for larger incidence rates for epilepsy in the female. It was further suggested that sex hormones and their interactions could provide some of the answers. The work of Schwartzkroin and Wyler (1980), among others, reinforced the notion that we all have a propensity to seizures given the right combination of precursors. The paradigm promoted in section 6.7 lends itself to further investigations. A lengthy retrospective examination of medical birth records could lead to the formation of a prospective cohort study of females to the end of their pubertal period. Specifically, one would be interested in finding out not only rates of epilepsy by type, but also seek relationships between physical/physiological events and subsequent seizure disorder. Inclusion of males in this cohort would add significant information through comparative research.

It may be argued that epilepsy rates are not high enough to warrant huge expenditures. Loiseau et al. (1983) suggest that some 40 per cent of female epileptics start seizuring around puberty. In addition, a smaller percentage go into various stages of remission around this time. In general this would mean some 4 persons per 1,000 of the population might benefit from this research. However this kind of reasoning represents a narrow view of the world. A large amount of

information is capable of being elicited from such investigation. To deny that such information might contribute not only to knowledge about epilepsy but also about other neurological disease, is to deny the complex inter-relationships of several variables operant in the central nervous system.

All of the preceding could lead to expansion in knowledge and, hopefully, the alleviation of suffering and reduction in some health care costs. However none of this will be possible without the direct co-operation of substantial numbers of people.

APPENDIX A

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Dear

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I am therefore sending you this letter to introduce him. Mr. Jacono has included an explanation of his study for you to read. If, after reading it, you decide that you would like to participate in his study, please follow his instructions.

Mr. Jacono is known to me and will be carrying out his research under the guidance of experts in the field.

Many thanks.

Yours truly,

TEF/lm

T.E. Feasby

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Mr. Jacono is known to me, and will be carrying out his research under the guidance of experts in the field.

Many thanks.

Sincerely,

W.T. Blume, M.D.

WTB:r

VICTORIA HOSPITAL CORPORATION – LONDON, ONTARIO
A. R. Thorlinnson, President

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Department of Clinical
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October 21, 1982

Dear M

Mr. John Jacono is a doctoral student in the department of Epidemiology and Biostatistics at the University of Western Ontario. He plans to do research about epilepsy in females and has asked me to help him find women who may be willing to help him.

I am, therefore, sending you this letter to introduce him. Mr. Jacono has included an explanation of his study for you to read. If, after reading it, you decide that you would like to participate in his study, please follow his instructions.

Mr. Jacono is known to me, and will be carrying out his research under the guidance of experts in the field.

Many thanks.

Sincerely,

Charles F. Bolton, M.D.,F.R.C.P.(C)

CFB/bt

SARAH A. STEWART, B.A., M.D., F.R.C.P (C)
NEUROLOGIST
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396 QUEENS AVE., SUITE 109
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18th October, 1982

Dear

Mr. John Jacono is a doctoral student in the department of Epidemiology and Biostatistics at the University of Western Ontario. He plans to do research about epilepsy in females and has asked me to help him find women who may be willing to help him.

I am therefore sending you this letter to introduce him. Mr. Jacono has included an explanation of his study for you to read. If after reading it, you decide that you would like to participate in his study, please follow his instructions.

Mr. Jacono is known to me, and will be carrying out his research under the guidance of experts in the field.

Many thanks,

Yours sincerely,

SARAH A. STEWART, M.D., F.R.C.P. (C)

SAS:mm

The University of Western Ontario

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Faculty of Medicine
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October, 1982.

Epilepsy Research Project

Dear M

This study is trying to find out, if some of the chemicals usually found in the body of females during the menstrual cycle, affect seizures. Let me say immediately that you will not be given any new drugs, nor will any drugs you are taking now be stopped. The study is scheduled to last for four menstrual cycles initially. Since each menstrual cycle is approximately one month long, the study will take about four months to complete. If you decide to participate, this is what you should expect to happen.

For the first 3 Menstrual Cycles

You will be asked to take your temperature daily, with a special thermometer for about three minutes each morning. This temperature must be recorded rectally as soon as you wake but before you get out of bed, smoke, eat or drink. In addition, you will be given a special seizure diary, in which you will keep a record of the number of seizures you have each day. I will be calling you once a week to get this information from you. At the end of each cycle, you will be asked to fill a questionnaire, and send it to me in a pre-stamped addressed envelope which I will supply.

For the 4th. Menstrual Cycle

In addition to taking your temperature and recording your seizures, a qualified nurse will visit you at home every other day in the morning to collect about two tablespoons of blood from you. This blood will be withdrawn before 10 a.m. and you must be in a fasting state until it is taken.

You will be shown how to take and register your temperature with the special thermometer, and how to record your seizures in the diary. At the end of these four cycles, all information gathered will be analysed. If the results prove encouraging, you may be asked to have blood withdrawn on alternate days again for another cycle. This will not take place for at least two months from the end of the previous collection period.

There is some discomfort from the taking of blood, the same as you feel when you have blood taken by your doctor or in the hospital. A risk of infection from the taking of blood also exists, but is very small.

All information about you, will be kept in a safe place. Any published material will not contain any particulars that may identify you to the reader.

Although I hope that you will participate, the decision is entirely yours. If you decide to not participate, or to withdraw,

during the study, this will in no way affect your future care.

If you decide to participate, will you please fill in and sign the consent form and return it to me in the enclosed envelope. I shall then contact you and fix a day to visit you.

Very Many Thanks

Sincerely

John Jacono

LETTER OF CONSENT

I _____, have read the letter of explanation about research of epilepsy in the female during the menstrual cycle. I understand what it says, and am awate of any risks involved. I understand that all information about me will be treated with the strictest confidence. I also understand that I can change my mind and drop out of the study later on if I wish. If I do drop out, this will not affect my future care.

I would like to participate in the study _____

(Subject)

Date:- _____ _____

(Witness)

The University of Western Ontario

Department of Epidemiology & Biostatistics
Faculty of Medicine
Kresge Building
London, Canada
N6A 5B7.

November, 1982.

Dear Ms.

I hope that by now you have received my letter inviting you to participate in the epilepsy research project.

If you have not made your mind up, would you please give the matter some thought; if you have made your mind up, would you please take a few minutes to answer the following questions and return them to me in the enclosed stamped, self addressed envelope.

Mark the box near the answer of your choice

1. I wish to participate in your project.
   My phone number is : _____
   Please call me. ☐

2. I do not wish to take part in your project, because:
   (a) I do not like being a research subject. ☐
   (b) I am already in a research project. ☐
   (c) I do not have the time to do all that you require of me. ☐
   (d) I do not like giving blood samples so often. ☐

Thank you for your co-operation

Sincerely

John Jacono

APPENDIX B.

The University of Western Ontario

Department of Epidemiology & Biostatistics
Faculty of Medicine
Kresge Building
London, Canada
N6A 5B7

October, 1983

Re: Epilepsy Research Project

Dear M

This study is trying to find out, if some of the chemicals usually found in the body of females during the menstrual cycle, affect seizures. Let me say immediately that you will not be given any new drugs, nor will any drugs you are currently taking be stopped. The study is scheduled to last for three menstrual cycles. Since each cycle is approximately one month long, the study will take about three months to complete. If you decide to participate, this is what you should expect to happen.

You will be asked to take your temperature daily, with a special thermometer which I will supply. This temperature must be taken for about three minutes as soon as you wake up each morning, but before you get out of bed, smoke, eat or drink. Because of the danger involved in having a glass thermometer in the mouth if a seizure should occur, the temperature should be taken rectally.

In addition I shall be supplying you with seizure diaries, in which you will record the number of seizures if any, that you experience each day. At the end of the three cycles, you will return the diaries and temperature records in a pre-stamped self addressed envelope that I shall provide.

I shall send you instructions on how to register your temperature, as well as how to fill in your seizure diary. Any information obtained will be treated with the strictest confidence, and no information published will identify you to the reader.

Although I hope that you will participate, the decision is entirely yours. If you decide to not participate, or to withdraw from the study, this will in no way affect your treatment or future care.

If you do decide to participate, would you please fill in and sign the consent form, and return it to me in the enclosed envelope. I shall then send you all the equipment necessary.

Many Thanks
Sincerely

John Jacono

LETTER OF CONSENT

I _____, have read the letter of explanation about research of epilepsy in the female during the menstrual cycle. I understand what it says, and am aware of any risks involved. I understand that all information about me will be treated with the strictest confidence. I also understand that I can change my mind and drop out of the study later on if I wish. If I do drop out, this will not affect my future care.

I would like to participate in the study _____

(Subject)

Date:- _____ _____

(Witness)

I.D. #

Epilepsy Research Project

Please fill in this form and return it to me with your first seizure diary.

(1) Date of Birth : _____

(2) Please list ALL THE PRESCRIBED MEDICATIONS you take, including the Birth Control Pill if you take it.

e.g.	Phenobarbitone	100 mgs.	Four times a day
(a)			
(b)			
(c)			
(d)			
(e)			
(f)			
(g)			
(h)			

Thank You for your Co-operation

I.D. #

Epilepsy Research Project

Instructions for using Basal Body Thermometer and recording your Basal Body Temperature

(1) Please make sure that the mercury is below 35.5° mark before taking your temperature.

(2) Leave the thermometer in the rectum for at least 3 minutes.

(3) Please mark down the temperature on your chart as soon as it is taken.

(4) Please remember to note down the time when the temperature was taken each morning.

(5) If the mercury does not quite reach a line on the thermometer, the correct reading is the next line below the top of the mercury.

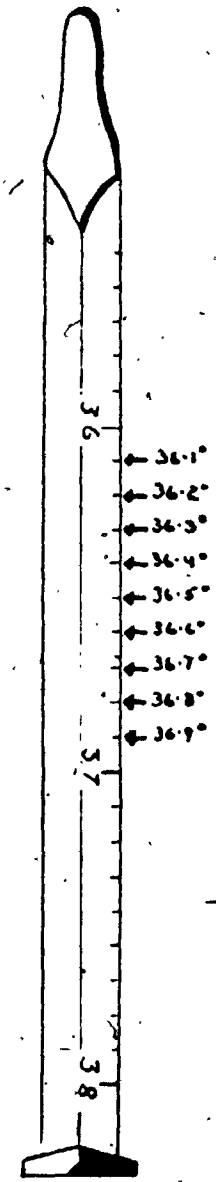
(6) If the mercury goes above the 38° mark, please mark it down on your chart as 38°+.

(7) When you get out of bed, wipe the thermometer clean and place it back in its cover to protect it.

(8) If The Thermometer Breaks :

Please call me at 433-8920 as soon as possible an I shall replace it the same day.

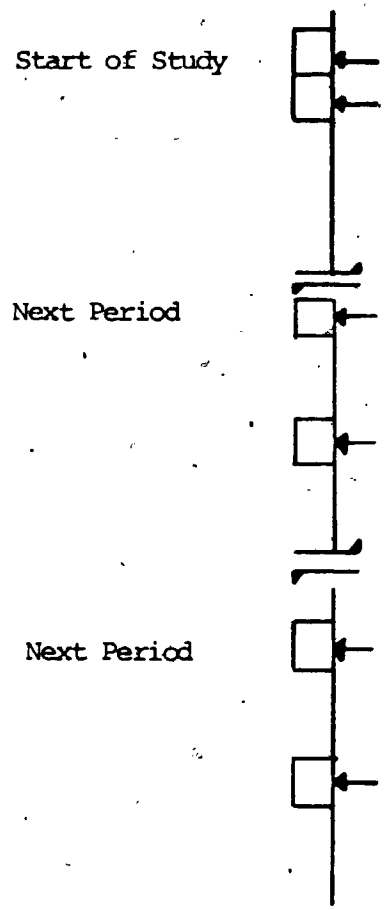
Thank you for your co-operation



I.D. #

Epilepsy Research Project

Instructions for Recording of Seizures and Temperature



Start of Study

First day of your period

Next Morning :
Start recording your temperature.
Start recording your seizures.

Next Period

1st. day of your period :
This morning is the last time you use your old temperature chart, and seizure diary.

Next Morning :
Start using a new temperature chart.
Start using a new seizure diary.

Next Period

1st. day of period :
This morning is the last time you use your old temperature chart,and seizure diary.

Next Morning :
Start using a new temperature chart.
Start using a new seizure diary.

Please remember to send me completed charts and diaries in envelopes provided.

Thank you for your co-operation

YOUR I.D. ______

Please put an "X" in this box each day you have your period

DATE	TIME	TEMPERATURE	

APPENDIX C

Ministry of Community and Social Services
Ontario

Oxford Regional Centre

P.O Box 310
Woodstock, Ontario
N4S 7X9
(519) 539-1251

Dear _____:

Mr. John Jacono, a Medical Researcher at the University of Western Ontario, is conducting a study of the effects of female hormones on epilepsy. In addition to normal subjects, he is trying to include some epileptic subjects from the Oxford Regional Centre.

Miss _____, your relative, qualifies as a possible subject. I am sending you Mr. Jacono's letter of explanation, and consent form. If you feel that you want to give permission for your relative to be included in this study, please sign the consent form, and return it in the enclosed stamped envelope. If you do not want your relative to participate, please discard this material.

This study is approved by the Oxford Regional Centre Research Committee.

Sincerely,

C.H. Lockwood, M.D.

jlm

The University of Western Ontario

Department of Epidemiology & Biostatistics
Faculty of Medicine
Kresge Building
London, Canada
N6A 5B7

February, 1983.

Research of Epilepsy in the Female

Dear M

It is possible that chemicals found in the blood of females during the menstrual cycle, affect seizures. This is a short explanation of what will take place in a proposed research study, aimed at finding out if there is indeed a connection between hormones and seizures.

Subject to permission being given by their next of kin, it is proposed that some residents of the Oxford Regional Centre may be used as subjects in this research. Miss _____ satisfies the conditions for entry into this study. Let me say immediately, that no new drugs will be given as part of this study; neither will any drugs your relative is currently taking for her epilepsy be witheld.

The research protocol calls for the recording of daily seizure activity, and the withdrawal of about two tablespoons of blood every other day for the duration of one menstrual cycle. There is also the possibility that blood may be withdrawn during a second cycle at a later date. There is some discomfort from the withdrawal of blood. A small risk of infection also exists.

Any information acquired will be held in the strictest confidence. Any published material will not contain any particulars that may identify your relative to the reader.

If you decide that you do not wish your relative to participate, or to withdraw your permission during the study, this will in no way affect your relative's care.

If you give permission for Miss _____ to paricipate, would you please sign the enclosed consent form, and return it to me in the enclosed stamped, self-addressed envelope.

Very Many Thanks

Sincerely

John Jacono

Research of Epilepsy in the Female

CONSENT FORM

I _____, being the next of kin of Miss _____, an inpatient at the Oxford Regional Centre at Woodstock Ontario, have read the letter of explanation about research of epilepsy in the female. I understand what it says and am aware of the risks involved. I understand that the researcher will have access to my relative's care file, and that all information will be treated with the strictest confidence. I also understand that I can change my mind and withdraw my permission later on if I wish. If I do withdraw my permission, this will in no way affect my relative's future care.

I hereby give my permission for the participation of MISS _____ in this study.

_____ _____

(Subject's Signature if Applicable) (Next of Kin's Signature)

_____

(Date)

APPENDIX D

Epilepsy Research Project

Instructions for Ward Personnel

On the day that the subject(s) on your ward start menstruating, would you please call your ward's Health Nurse and give her the following informatio:

(a) Tell her you are calling about a subject involved in the epilepsy research project.

(b) Identify the subject by name and the ward in which she resides.

(c) Tell the health when menstrual flow was first noticed.

(d) The following morning please make sure that the subject does not eat until after blood withdrawal. Please give her prescribed medications as usual.

(e) The subject will have blood withdrawn while in a fasting state on alternate days for one menstrual cycle. Blood will be withdrawn before 10.30 a.m.

(f) Please keep a close watch on, and record every seizure on the diaries provided for the length of the study cycle.

(g) Please record ALL medications given to the subject during the study cycle, including any analgesics and laxatives.

(h) If the subject should inadvertently eat before blood collection, have the blood collected regardless, but Tell the Laboratory Tech. that the subject had eaten and the approximate time when this happened.

(i) The first day of the next menstrual cycle, please call the ward's health nurse again and repeat instuctions A to C. Blood collection for that subject will stop on that day.

Thank you all again for your co-operation. Please do not hesitate to call me for any reason regarding the study at the phone numbers provided.

John Jacono

Epilepsy Research Project

Instructions for Laboratory Technicians

Blood withdrawal will start on or after April 29th. Please call the switchboard operator every morning who will give you the names of subjects eligible for blood collection on that day.

(a) The day following the start of menstrual flow will be the first blood collection day. Blood collection will continue on alternate days to the first day of the next menstrual flow.

(b) Subjects should be in a fasting state, and blood must be collected before 10.30 a.m.

(c) If the subject should happen to have eaten prior to blood collection, please withdraw blood regardless but note food intake and time in the "PROBLEMS ?" section of your collection log.

(d) Please collect blood for ionized calcium assay (i.e. in the Royal Blue Top Vacutainer) last. Make sure that the tube is filled to capacity to preserve anaerobicity.

(e) Please centrifuge blood for 10 minutes before extracting serum without removing Vacutainer cap. Freeze serum as soon as possible after extraction.

(f) Ward staff will report the start of the next menstrual flow through the arranged channels. The switchboard operator will give you this information.

(g) I shall collect frozen serum on a weekly basis. Please do not hesitate to call me at the numbers provided if you have any concerns.

Thank you all

John Jacono

APPENDIX E

Estimation of Correction Factor (C)

For Clustering Effect in Repeated Measurements

The estimation of the proportion of seizures that occur in a particular stage of the menstrual cycle is simply the number of seizures in that particular stage of the cycle, divided by the total number of seizures exhibited during the cycle. The usual estimated variance of $\hat{p}$ is :

$$V = \hat{p}(1 - \hat{p}) / n$$

where n is the total number of seizures. This estimator is valid only if all observations are independent. This is not the case here, since each subject contributes several observations to the data.

In such a situation, as certain subjects experience a higher or lower incidence of seizures than the average, the estimated (V), attributes the same weight to five seizures in one subject as it does to one seizure in each of five subjects. As a result, (V) is an underestimation of the true variance of $\hat{p}$.

Brier (1980), has shown that the true variance is :

$$\text{Var}(\hat{p}) = \frac{(\bar{m} + k)}{(1 + k)} V$$

where :

(1) $\bar{m}$ is the mean cluster size.
(2) k is a parameter between 0 and infinity describing the extent of clustering. When k is large, little or no clustering is taking place; conversely, when k approaches 0, strong clustering is evident.

Brier demonstrated that the correction factor :

$$C = \frac{(\bar{m} + k)}{(1 + k)}$$

can be extended to hypothesis testing. Thus the usual X^2 Goodness-of-Fit statistic, can be adapted to a X^2/C which has an approximate chi-square distribution.

For each cluster of size m, Brier (1980) suggested a method of estimating the correction factor (C), where:

$$\hat{C}_m = \frac{1}{(N_m - 1)(r - 1)} \sum_{i=1}^{N_m} \sum_{j=1}^{r} \frac{(X_{ij} - m\hat{p}_j)^2}{m\hat{p}_j}$$

where :

(1) N_m is the number of clusters of size m.

(2) r is the number of response categories,

(3) $\hat{p}_j$ is the estimated probability of a $j^{th.}$ category response.

(4) X_{ij} is the number of $i^{th.}$ cluster members falling in the $j^{th.}$ category.

For each m it is now possible to compute :

$$\hat{k}_m = \frac{m - \hat{C}_m}{\hat{C}_m - 1}$$

and produce a weighted mean of the $\hat{k}_m$s:

$$\hat{k} = \frac{\Sigma_m \hat{k}_m \, mN_m}{\Sigma mN_m}$$

thus $$\hat{C} = \frac{\bar{m} + \hat{k}}{1 + \hat{k}}$$

When two response categories are available, k may be shown to be:

$$k = (1 - \rho)/\rho$$

where ρ is the intra-cluster correlation coefficient used in the analyses of continuous data and equal to :

$$\frac{\sigma_A^2}{\sigma_T^2}$$

This leads to a new expression for C :

$$C = 1 + (\bar{m} - 1)\rho$$

if ρ is estimated under the assumption that each dichotomous (0,1) datum is continuous and the analysis of variance methods of Snedecor and Cochran (1980) methods are applied.

In the dichotomous case therefore, there are two estimates of C. That based on Brier's moment estimation:

$$\hat{C}_m = \frac{\bar{m} + \hat{k}}{1 + \hat{k}}$$

and the ANOVA estimator:

$$\hat{C}_A = 1 + (\bar{m} - 1)\,\hat{\rho}_A$$

Donald (1984), in a simulation study found that $\hat{C}_m$ tends to overestimate the true C, in small samples and recommended the use of $\hat{C}_A$ for dichotomous responses. Donald also found that $\hat{C}_A$ was a less biased estimator of the correction factor.

APPENDIX F

Appendix F

Five non-institutionalized female epileptics, all satisfying the eligibility criteria in section 4.2.1, had contributed data prior to their removal from the study. It may be recalled that termination from participation was necessitated by a low response and the wide geographic distribution of these subjects. Each subject had followed the data collection protocol described in section 4.2.2.4. Thus each subject contributed a basal body temperature chart and a seizure diary for three consecutive menstrual cycles. Distribution of seizures by day and cycle stage is presented in Figure 12. The day preceeding thermal shift associated with ovulation was assigned to day 0. All determinations were forced into a thirty day cycle on the same basis as the study subjects. Thus direct comparison with seizure distribution exhibited by the study subjects became possible. A goodness of fit χ^2 between seizures exhibited by the non-institutionalized subjects and the study subjects by cycle stage yielded a χ^2 with 2 df. of 5.99, $P = 0.05$. Adjustment for clustering effect would have significantly lowered the χ^2 value rendering it statistically not significant. It may then be argued that seizure distribution in these non-institutionalized subjects closely approximated that exhibited by the study subjects. This provides further support for the distribution of seizures encountered during the menstrual cycle of the study subjects.

Seizure Distribution by Cycle day / Cycle Stage

n = 5 non-institutional subjects each contributing three menstrual cycles and forced into a 30day cycle.

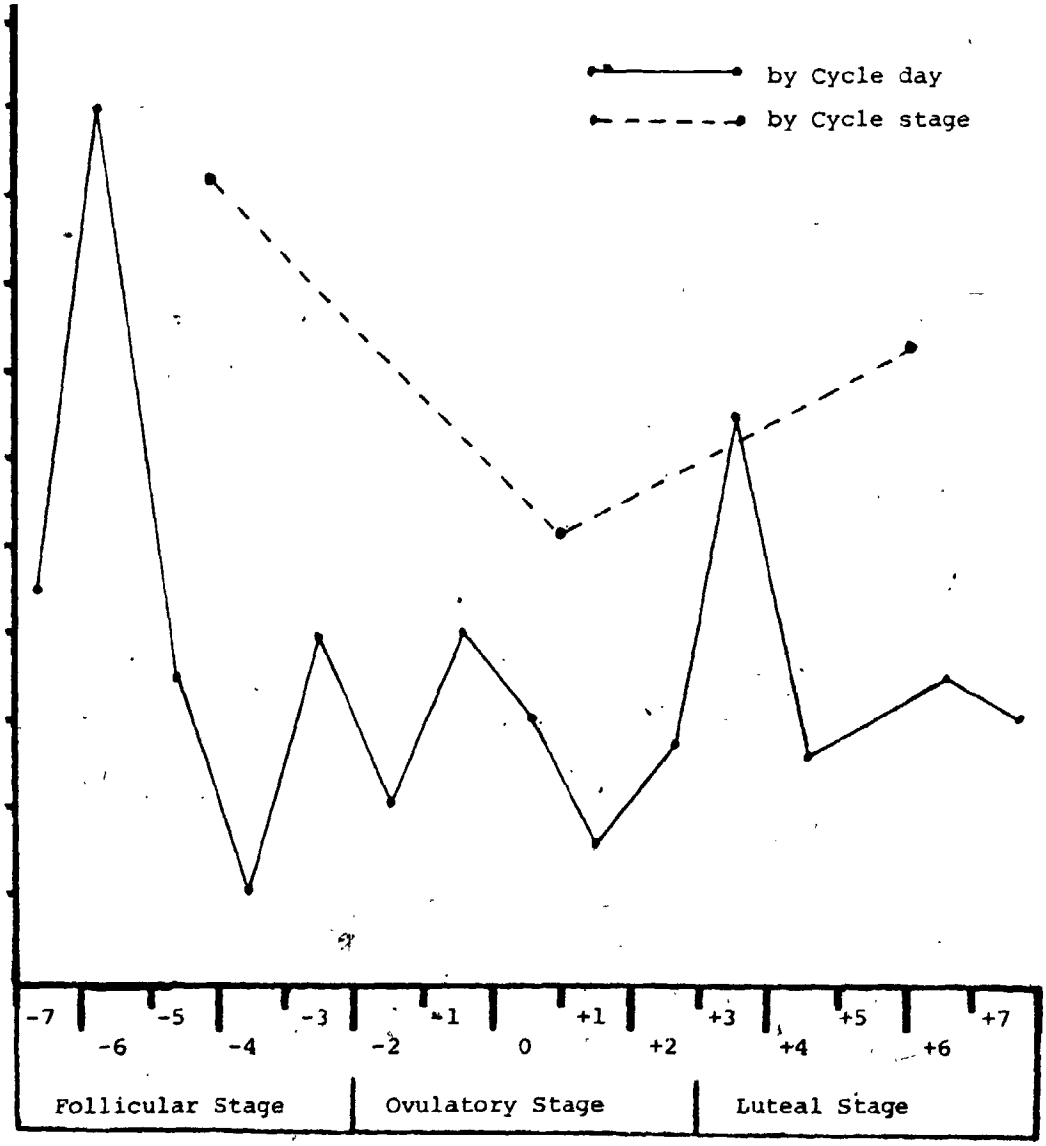


Figure 12.

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